Transcriptome-wide identification of differentially expressed genes and long non-coding RNAs in nitroglycerin-tolerant rat aorta

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Abstract: Tolerance to nitroglycerin (GTN) greatly limits its long-time application, and the underlying mechanism remains largely unexplored. In the present study, we aimed to investigate the comprehensive changes in the transcriptome of rat aorta tolerant to GTN by analyzing lncRNA expression. We employed the RNA sequencing (RNA-seq) technique to identify mRNAs and lncRNAs. Ingenuity pathway analysis (IPA) was used for pathway and functional analysis. RT-qPCR was used to validate the RNA-seq results. We identified 22 788 genes (reads per kilobase million [RPKM] > 0.1, 14 720 protein-coding genes and 4408 lncRNAs), including 115 differentially expressed (DE) mRNAs (65 up-regulated and 50 down-regulated) and 104 DE lncRNAs (56 up-regulated and 48 down-regulated), in GTN-tolerant aortas. IPA revealed the inhibition of a canonical pathway "Signaling by Rho Family GTPases" and alteration in six upstream regulators. Functional analysis showed that 11 genes were related to "disorder of blood pressure". We predicted the *cis*-target genes of DE lncRNAs by the analysis of their neighboring genes. The results revealed the 28 DE lncRNAs adjacent to the 26 protein-coding genes. Many DE mRNAs and *cis*-target genes of DE lncRNAs have been implicated in the regulation of blood pressure or cell contraction. These results suggested that the dysregulated mRNAs and lncRNAs contributed to the development of GTN tolerance and could serve as potential targets to prevent and reverse GTN tolerance.

Keywords: Transcriptome; Nitroglycerin; Tolerance; LncRNA; Aorta

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1. Introduction

Nitroglycerin (glyceryltrinitrate, GTN) is a commonly used drug for the treatment of angina pectoris, heart failure, acute myocardial infarction, and other diseases. However, the long-term or repeated application of GTN may reduce or obliterate its biological effects, resulting in GTN tolerance. To avoid GTN tolerance, the most commonly employed strategy involves the provision of a daily GTN-free interval[1]. However, this method is associated with an increased risk of ischemic events during the GTN-free interval[2]. Besides, GTN tolerance may lead to platelet aggregation and endothelial dysfunction and aggravate the prognosis of patients with coronary heart disease[1].

However, the exact cause of GTN tolerance is still incompletely understood, some mechanisms, such as inhibition of GTN activation, production of reactive oxygen, inactivation of soluble guanylylcyclase (sGC), and impediment of cyclic guanosine monophosphate (cGMP) pathway, are thought to play important role[2–5]. Recent reports have suggested the involvement of microRNAs in GTN tolerance[6].

Long non-coding RNAs (lncRNAs) are a diverse class of RNAs that are engaged in numerous biological processes[7]. LncRNAs function through different mechanisms,

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such as chromatin modification, *cis/trans* regulation of target genes, and post-transcriptional, translational, and post-translational regulation. These molecules interact with proteins, DNAs, or other RNAs[7]. Several lncRNAs, such as SENCR[8–10], lncRNAp21[11], MYOSLID[12], SMILR[13], and lncRNA TUG1[14], have been linked to vascular functions. However, the role of lncRNAs in the development of GTN tolerance remains largely unclear.

Here, we used a rat model to study the transcriptomic changes in the aorta tissue by RNA sequencing to explore the variations in mRNA and lncRNA expression patterns in response to GTN tolerance. Our results suggested that the dysregulated mRNAs and lncRNAs contributed to GTN tolerance, and some of these molecules could serve as good targets to overcome GTN tolerance.

2. Materials and methods

2.1. GTN-tolerant rat model

Male Sprague-Dawley (SD) rats (180–200 g) were given GTN (100 mg/kg subcutaneously) or its vehicle (ethanol) three times a day for 3 d as previously described[15–17]. Vascular tolerance to GTN was confirmed with carotid artery catheterization on day 4, approximately 12 h after the last injection. The rats were anesthetized with an intraperitoneal injection of 60 mg/kg sodium pentobarbital. The left carotid artery was isolated, and a catheter connected to a pressure transducer was inserted to monitor blood pressure (BP). The right jugular vein was isolated and catheterized for GTN administration. BP responses to intravenous administration of 10 μg/kg of GTN were recorded by PowerLab system (AD intruments, Australia). Then thoracic aorta was excised, washed with ice-cold normal saline, and preserved in RNAlater (Sigma, USA).

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal protocols were reviewed and approved by the ethics committee of the Hunan University of Medicine.

2.2. RNA library construction and sequencing

Total RNA was isolated using miRNeasy Mini Kit (Qiagen, USA) following the manufacturer's procedure. The total RNA quantity and purity were analyzed using Aligent 2100 Bioanalyzer (Agilent, USA) with a RIN number > 7.0. Four RNA samples from each group were used for processing and sequencing. Ribosomal RNA was depleted using EpicentreRibo-Zero Gold Kit (Illumina, San Diego, USA). Following purification, the poly(A)- or poly(A)+ RNA fractions were fragmented into small pieces using divalent cations under elevated temperatures. Then the cleaved RNA fragments were reversely transcribed to create the final cDNA library following the protocol, and the average insert size for the paired-end libraries was 300 bp (± 50 bp). Finally, we performed the 2 × 150 bp paired-end sequencing on an IlluminaHiseq 4000 machine (Lc-Bio, China) following the vendor's recommended protocol. The Gene Expression Omnibus (GEO) accession code for the data in this manuscript was GSE121924.

2.3. Transcript assembly

Sequence quality was assessed using FastQC (v0.10.1) after the removal of adaptors, low-quality bases, and undetermined bases using Cutadapt (v1.10)[18]. Bowtie2 (v2.0.0)[19] and Topaht2 (v2.0.4)[20] were used to map the reads to the rat genome (Rnor_6.0.83). The mapped reads of each sample were assembled using StringTie (v1.3.0)[21]. Then, all transcriptomes from samples were merged to reconstruct a comprehensive transcriptome using Perl. The expressions of all transcripts were calculated using StringTie[21] and Ballgown (v2016.09.29)[22].

2.4. LncRNA identification

The coding potential of transcripts was predicted using CPC (v0.9-r2)[23] and CNCI (v2.0)[24] after discarding the transcripts that overlapped with known mRNAs or those shorter than 200 bp. The transcripts with a CPC score < −1 and CNCI score < 0 were eliminated. The remaining transcripts were identified as lncRNAs.

2.5. Different expression analysis of mRNAs and lncRNAs

The expressions of mRNAs and lncRNAs were calculated with StringTie (v1.3.0) using fragments per kilobase per million mapped reads (FPKM)[25]. The differentially expressed (DE) mRNAs and lncRNAs were selected based on $|\log_2$ (fold change)$|$ ($\log_2$FC) > 1 and statistical significance ($P < 0.05$) with R package-Ballgown[22].

2.6. Target gene prediction

To explore the function of lncRNAs, we predicted the *cis*-target genes of lncRNAs. LncRNAs may play a *cis* role in neighboring target genes. In the present study, coding genes in 100 000 upstream and downstream were selected by Python script.

2.7. Ingenuity pathway analysis (IPA)

The canonical pathway analysis, the upstream regulator analysis, and downstream effect (diseases and functions) analysis were carried out by subjecting the DE genes ($P < 0.05$ and absolute fold-change > 1) to the IPA tool (Qiagen, Duesseldorf, Germany). Activation and inhibition of the pathway were accessed with *Z*-score[26]. The right-tailed Fisher's exact test was used to calculate statistical significance, and a value of $P < 0.05$ indicated statistical significance and non-random association.

2.8. Gene expression validation by reverse-transcription quantitative polymerase chain reaction (RT-qPCR)

RNA ($n = 5$ in each group) was reversely transcribed using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies) at 37 °C for 60 min and 95 °C for 5 min. The obtained cDNA was subjected to qPCR using TaqMan Fast Advanced Master Mix (Thermo Fisher, USA) and TaqMan Assay (Thermo Fisher, USA) on LightCycler 480 Real-Time PCR System (Roche, USA). PCR cycling conditions were as follows: 50 °C for 2 min, 95 °C for 20 s, 40 cycles of 95 °C for 3 s and 60 °C for 30 s, followed by preservation at 4 °C. The expressions were calculated using the $2^{-\Delta\Delta Cq}$ method, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was selected as the endogenous reference gene. The unpaired two-tailed Student's *t*-test was used to calculate the differences between the two groups. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. LncRNA and mRNA profiles after GTN tolerance

After administration of GTN for 3 d, the vasorelaxant response to GTN disappeared. We conducted RNA-seq analysis (Fig. 1A) and found that the reads of each sample were about 100 million, and Q30 of the clean data from all samples was above 93% (data not shown). More than 80% of the reads were mapped to the reference genome (Rnor_6.0.83).

A total of 22 788 genes were identified (defined as RPKM > 0.1), and 85.37% were expressed in both groups (Fig. 1B). Of all expressed genes, 14 720 were protein-coding genes, while 4408 genes corresponded to lncRNAs. Further inspection revealed that the expressions of the protein-coding genes were more common compared with the lncRNAs in the aorta (93.24% *vs.* 64.34%).

These unique lncRNAs (35.66%) and protein-coding genes (6.76%) expressed in the aorta might be the result of GTN tolerance (Fig. 1B).

For further analysis, genes with $P < 0.05$ and $|\log_2 FC| > 1.0$ were considered significantly different. A total of 115 DE mRNAs were found in GTN-tolerant aortas, including 65 up-regulated and 50 down-regulated mRNAs (Fig. 2A). A total of 104 DE lncRNAs were found, including 56 up-regulated and 48 down-regulated lncRNAs (Fig. 2B).

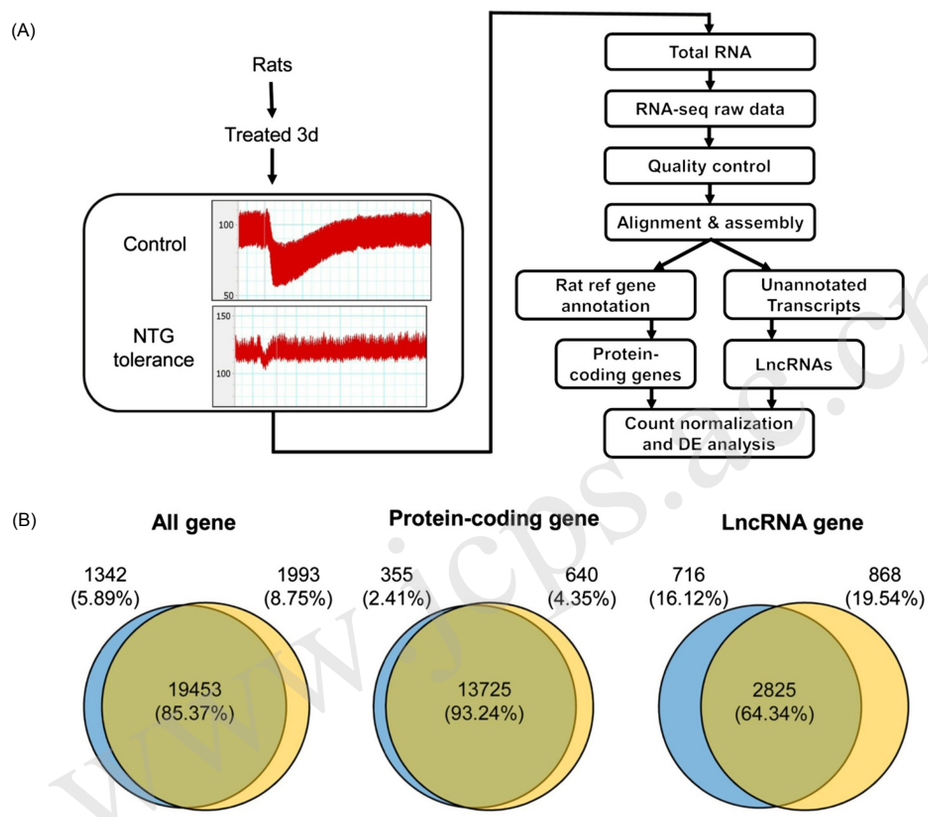

Figure 1. Experimental flow and transcriptome landscape. (A) Experimental flow. (B) Venn diagrams depicting the overlap of the expressed genes (RPKM > 0.1) in the aortas of control rats (blue) and GTN-tolerant rats (yellow).

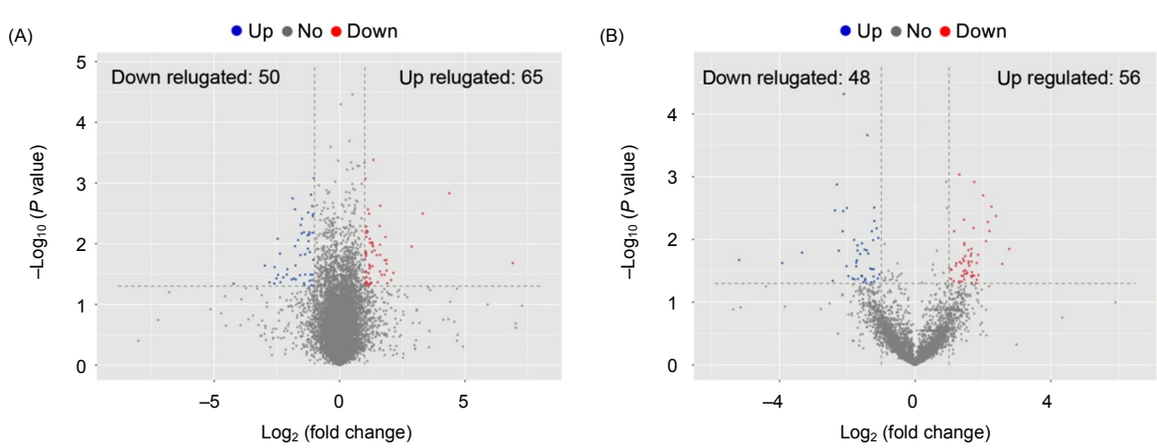

Figure 2. DE genes after GTN tolerance. Volcano plots of DE mRNAs (A) and DE lncRNAs (B). $|\text{Log}_2 FC| = 1.0$ was used as a threshold.

We selected nine protein-coding genes (four oxidative stress genes [*Maff*, *Nox4*, *Apoe*, and *Timp3*], three genes related to cell contraction [*Gucy1b2*, *Myl9*, and *Ckm*], one [*Corin*] involved in the regulation of BP, and one [*Dtx*] as a post-translational regulator) and four lncRNA genes (randomly) for further RT-qPCR validation, indicating that the sequence results were reliable (Table 1).

3.2. IPA of the DE mRNAs

In the canonical pathway analysis, one pathway "Signaling by Rho Family GTPases" was inhibited (*Z*-score = −1.342) (Table 2A). IPA predicted the GTN tolerance-mediated inhibition of two upstream regulators (ZBTB16 and VCAN) and activation of four upstream regulators (TNF, CSF1, TGFB1, and cholesterol) (|*Z*-score| ≥ 2) (Table 2B). Functional analysis in cardiovascular disease showed that "Aneurysm" and "Disorder of BP" were inhibited (|*Z*-score| ≥ 1) (Table 2C), and *Apoe*, *Ccl2*, *F2rl1*, and *Ccl7* were the most frequently observed genes (7, 6, 5 and 5 times, respectively). The change in the expression of *Apoe* is shown in Table 2, while that of the other three genes was validated with RT-qPCR (Table 3).

Table 1. RNA-seq and RT-qPCR results of the selected genes in the aorta of GTN-tolerant rats as compared with the controls.

Gene name	Gene description	Mean FPKM		Log_2FC	*P* value	qRT-PCR	
		CON	GTN			Log_2FC	*P* value
Maff	MAF bZIP transcription factor F	1.70	11.78	4.37	0.0015	1.29	0.0861
Apoe	Apolipoprotein E	12.79	43.17	2.87	0.0111	1.02	0.0004
Timp3	TIMP metallopeptidase inhibitor 3	43.60	116.20	1.87	0.0277	0.51	0.0161
Gucy1b2	Guanylatecyclase 1 soluble subunit beta 2	0.32	1.08	1.13	0.0027	1.21	0.0116
Corin	Corin, serine peptidase	3.54	8.04	1.08	0.0354	0.96	0.0091
Nox4	NADPH oxidase 4	0.66	1.83	1.03	0.0284	0.87	0.0440
Myl9	Myosin light chain 9	152.17	92.68	−1.09	0.0397	−0.74	0.0285
Ckm	Creatine kinase, M-type	9.78	5.06	−1.27	0.0073	−0.69	0.0029
Dtx1	Deltex E3 ubiquitin ligase 1	4.79	2.22	−1.78	0.0110	−1.18	0.0023
MSTRG.4100	lncRNA	4.36	3.65	−1.23	0.0297	−0.89	0.0147
MSTRG.7048	lncRNA	1.80	0.29	−2.31	0.0013	−1.03	0.0376
MSTRG.8058	lncRNA	2.05	7.81	1.41	0.0122	1.14	0.0291
MSTRG.14197	lncRNA	0.23	1.06	1.04	0.0297	0.98	0.0415

Table 2. IPA of the DE mRNAs.

A) Ingenuity canonical pathways analysis

Ingenuity canonical pathway	−Log (*P* value)	*Z*-score	Molecules
Signaling by Rho family GTPases	2.25	−1.342	MYL9, PIK3R3, NOX4, CIT, CDH13

B) Upstream regulator analysis

Upstream regulator	Activation state	Activation *Z*-score	*P* value of overlap	Target molecules in dataset
ZBTB16	Inhibited	−2	0.000426	CCL2, CCL7, DTX1, F2RL1, TSC22D1
VCAN	Inhibited	−2.219	0.000759	APOE, Ccl2, CDH13, MX1, PRELP
TNF	Activated	2.413	0.00000264	ADAMTS8, APOE, BHLHE40, CCL2, CCL7, CDH13, CHI3L1, CKM, CLEC11A, CRLF1, CX3CR1, CYP26B1, EFNB2, F2RL1, HES1, MAFF, MX1, NOX4, SDC1, SLC1A3, TIMP3, TM4SF1, TXN2
CSF1	Activated	2.213	0.0000249	APOE, CCL2, CCL7, F2RL1, GOT2, SLC1A5, XBP1
TGFB1	Activated	2.611	0.000305	APOE, BHLHE40, CCL2, CCL7, CHI3L1, CKM, COL6A2, CORIN, CX3CR1, DDX21, ESAM, F2RL1, HES1, NOX4, SDC1, TIMP3, TSC22D1, TUBB4B, XBP1
Cholesterol	Activated	2.214	0.000433	APOE, Ccl2, Ccl7, SLC1A3, TIMP3, XBP1

C) Functions analysis in cardiovascular disease

Functions annotation	*P* value	Activation *Z*-score	Molecules
Aneurysm	0.00127	−1.176	APOE, EFNB2, SDC1, TIMP3
Disorder of blood pressure	0.00423	−1.02	APOE, CDH13, CORIN, F2RL1, HES1, NOX4, NPY, PRELP, SPCS2, TXN2, XBP1

Table 3. RNA-seq and RT-qPCR results of the top genes in IPA.

Gene name	Gene description	Mean FPKM		Log_2FC	*P* value	RT-qPCR	
		CON	GTN			Log_2FC	*P* value
Ccl2	C-C motif chemokine ligand 2	0.58	2.14	1.79	0.0325	0.81	0.0098
F2rl1	F2R-Like trypsin receptor 1	92.68	152.17	1.01	0.0365	0.53	0.0116
Ccl7	C-C motif chemokine ligand 7	0.17	1.08	1.20	0.0096	0.88	0.0098

Table 4. Lists of lncRNAs within a reference gene intron or that overlapped with a reference gene on the opposite strand.

LncRNA gene	FPKM		Log_2FC	*P* value	Gene	Class code	FPKM		Log_2FC	*P* value
	Con	GTN					Con	GTN		
MSTRG.11899	0.87	1.61	1.14	0.0462	RT1-T24-3	x	2.53	4.47	1.61	0.0152
MSTRG.11977	2.94	0.03	−3.34	0.0162	RT1-CE6	x	ND			
MSTRG.16540	0.56	1.20	2.77	0.0142	SNORA73	x	0.00	0.98	0.13	0.455
MSTRG.5937	0.55	1.14	1.79	0.0496	Mir3120	x	0.05	0.228	0.58	0.276
MSTRG.7194	1.47	0.94	−1.24	0.0103	Samd4a	x	4.49	7.25	0.52	0.3835
MSTRG.11146	0.19	0.83	1.86	0.037	Fnip2	i	4.19	4.89	0.03	0.9567
MSTRG.11902	0.34	1.07	1.47	0.0183	RT1-T24-1	i	0.74	0.62	−0.49	0.071
MSTRG.13413	0.96	0.44	−1.74	0.0233	Ehd4	i	2.98	4.18	0.10	0.6691
MSTRG.14381	0.35	0.18	1.21	0.0239	Bmt2	i	1.71	2.19	0.06	0.744
MSTRG.15163	0.79	0.61	−1.70	0.015	Zfp384	i	3.11	4.61	0.07	0.7821
MSTRG.16440	0.42	1.21	2.57	0.0245	Ago3	i	3.74	5.31	−0.22	0.040
MSTRG.16955	0.16	1.24	2.19	0.0073	Eml4	i	2.36	3.31	0.52	0.143
MSTRG.17477	0.15	0.51	1.57	0.0148	Mdga2	i	1.92	2.27	−0.36	0.120
MSTRG.18153	0	1.49	1.30	0.0009	Pym1	i	1.71	3.58	1.04	0.280
MSTRG.19477	2.86	0	−2.11	0	Cdc37	i	7.61	9.61	0.10	0.5829
MSTRG.20491	1.25	1.95	1.67	0.0394	Stac	i	1.29	1.37	0.47	0.272
MSTRG.2333	1.43	0	−1.21	0.0051	Fas	i	2.28	3.61	0.20	0.6883
MSTRG.2526	0.48	0.58	1.53	0.0329	Mxi1	i	2.04	3.78	0.63	0.526
MSTRG.3890	0.16	0.96	1.31	0.0228	Dcakd	i	1.39	2.02	0.84	0.077
MSTRG.5860	0.60	0.21	−1.57	0.0386	Abl2	i	1.63	2.30	0.83	0.166
MSTRG.6484	0	1.13	1.15	0.0074	Ppat	i	1.70	1.69	−0.29	0.4569
MSTRG.7017	0.17	0.71	1.69	0.0375	Mtif2	i	0.66	1.11	0.10	0.551
MSTRG.7043	4.98	6.57	−1.30	0.0483	Kcnma1	i	3.24	3.96	−0.25	0.4801
MSTRG.7045	3.49	3.46	−1.74	0.0268	Kcnma1	i	3.24	3.96	−0.25	0.4801
MSTRG.7048	1.8	1.85	−2.31	0.0013	Kcnma1	i	3.24	3.96	−0.25	0.4801
MSTRG.7322	1.09	1.47	−1.80	0.0101	Zmym2	i	ND			
MSTRG.7918	0.65	0.27	−1.69	0.0426	Fbxo8	i	2.17	2.88	0.13	0.712
MSTRG.8217	0.75	0.27	−1.85	0.042	Irs2	i	0.52	1.24	−0.18	0.6638
MSTRG.8589	1.37	1.58	−1.97	0.0385	Tdp2	i	3.05	4.76	−0.41	0.231
MSTRG.8985	0.6	0.72	−1.44	0.0498	Etl4	i	2.32	3.08	0.20	0.4866
MSTRG.9736	0.99	0.50	−2.37	0.0034	Siah1	i	1.23	1.38	−0.64	0.166
MSTRG.9882	1.01	1.35	−1.48	0.045	Cbfb	i	1.75	2.43	−0.20	0.4492
MSTRG.9989	2.49	2.29	−1.09	0.0095	Zfhx3	i	7.53	8.02	−0.23	0.326

LncRNAs and neighboring genes that are < 100 kb. ND: not detected. i: a transfrag falling entirely within a reference intron; x: exonic overlap with reference on the opposite strand.

3.3. Analysis of lncRNA target genes

To predict the *cis*-target genes of DE lncRNAs, the coding genes in the region 100-kb upstream and downstream were selected. The results showed that 52 DE lncRNAs ($P < 0.05$, $|\log_2FC| > 1.0$) were near 113 genes, 26 DE lncRNAs were present within a reference gene intron, and five DE lncRNAs showed an exonic overlap with a reference gene on the opposite strand (Table 4). Further inspection revealed that only one target gene, *RT1-T24-3*, was differently expressed in the mRNA profile ($P < 0.05$, $|\log_2FC| > 1.0$, Table 4).

4. Discussion

The vasodilatory action of GTN is dependent on the release of vasodilatory molecules, especially nitric oxide (NO). In general, GTN acts as a NO donor and causes vasodilation *via* the sGC-cGMP-PKG pathway[1]. Besides, GTN regulates vasodilation through an epigenetic way[27].

One major problem with the long-term use of GTN is the development of tolerance. To date, several hypotheses have been proposed to explain the development of GTN tolerance, and many of these suggest the effect at the post-translational level, such as inhibition of aldehyde dehydrogenase 2 enzyme activity, enhancement in phosphodiesterase activity, desensitization of sGC, and activation of RhoA/Rho kinase pathway[1–4]. LncRNAs are known to regulate protein functions through their direct interactions with the target proteins. Hence, we supposed that lncRNAs were involved in the development of GTN tolerance.

In recent years, the role of noncoding RNAs in cardiovascular diseases has received widespread attention[28,29]. In the present study, we provided a comprehensive analysis of the transcriptome in the aorta by specifically focusing on lncRNAs. As a result, we found that the aorta transcriptome profile of GTN-tolerant rats was significantly different from that of the control rats.

Many of the DE protein-coding mRNAs were associated with GTN tolerance, cell contraction, and regulation of BP. We chose nine genes for RT-qPCR validation. Among these genes, four were related to oxidative stress (*Maff*, *Nox4*, *Apoe* and *Timp3*), which were up-regulated in GTN-tolerant rats. These genes might account for the production of reactive oxygen species (ROS), which were thought to be the key mediators of GTN tolerance. Besides, three genes (*Gucy1b2*, *Myl9*, and *Ckm*) related to cell contraction, one gene (*Corin*) involved in the regulation of BP, and one (*Dtx1*) regulator of Notch signaling that plays a key role in cardiovascular development and diseases were detected. All of these validated genes, except for *Nox4* (reported in a previous study[15]), have not been previously associated with GTN tolerance and need to be investigated in the future. We also found that the mRNA profile was substantially different as compared with other cDNA microarray analyses[15,30], probably owing to the use of the new reference genome as well as the high sensitivity of RNA-seq.

We used IPA to explore the pathways related to the DE mRNAs. Canonical pathway analysis revealed the inhibition of the Rho signaling pathway (involving five genes). The activation of the RhoA/Rho-associated protein kinase (ROCK) pathway is known to contribute to vasoconstriction *via* the inhibition of myosin light-chain phosphatase, which is a potential mechanism underlying GTN tolerance. However, the inhibition of the Rho signaling pathway observed in the present study might be the result of feedback inhibition. The activation of the Rho pathway leads to the inhibition of the expression of the related genes. Upstream regulator analysis revealed the inhibition of two regulators (ZBTB16 and VCAN) and activation of four (TNF, CSF1, TGFB1, and cholesterol) regulators. Further studies are warranted to evaluate the role of these upstream regulators in GTN tolerance. Functional analysis showed that 11 genes were related to “disorder of BP” and four genes were associated with “aneurysm.”

In IPA analysis, *Apoe* was the most frequently observed gene (7 times), followed by *Ccl2* (6 times), *F2rl1* (5 times), and *Ccl7* (5 times). These genes might account for GTN tolerance. Apolipoprotein E (APOE) is essential for the normal catabolism of triglyceride-rich lipoprotein constituents and contributes to the development of several cardiovascular diseases. However, it remains unknown whether APOE is involved

in the contraction of vascular smooth muscle cells and GTN tolerance. Chemokine (C-C motif) ligand 2 (CCL2) and CCL7 are important mediators of inflammation, which are involved in atherosclerosis, vascular dysfunction, and secondary hypertension. However, these markers have not been confirmed to be directly involved in the contraction of vessels[31,32]. F2R-like trypsin receptor 1 (F2RL1) encodes protease-activated receptor 2 (PAR2). It has been reported that the activation of the receptor can stimulate vascular smooth muscle relaxation, dilation of blood vessels, and BP reduction through the induction of NO production *via* the phosphorylation of endothelial nitric oxide synthase[33–35].

We identified 4408 lncRNAs (RPKM > 0.1) and found 104 DE lncRNAs ($|\log_2 FC| > 1$, $P < 0.05$). Considering the small number of studies and the lack of the comprehensive annotation of rat lncRNAs, the exact structures, and functions of these lncRNAs warrant further investigation. In the present study, we predicted the *cis*-target genes of DE lncRNAs through the analysis of their neighboring genes. The results showed that only one target gene, *RT1-T24-3*, was differently expressed. However, there was no evidence that *RT1-T24-3* was related to vascular diseases and GTN tolerance. Although most lncRNA-targeted genes showed no change in their expression levels, the lncRNAs still functioned by directly regulating the expression and/or function of the proteins encoded by these genes, especially, those implicated in the regulation of BP or cell contraction. For instance, *Kcnma1* (MaxiK) encodes calcium-activated potassium channel subunit alpha-1, also known as large-conductance calcium-activated potassium channel, subfamily M, alpha member 1 (KCa1.1). KCa1.1 is essential for the control of smooth muscle tone[36–38] and may serve as a target for the endothelium-derived hyperpolarizing factor (EDHF) in some blood vessels[39].

Studies have demonstrated the ability of lncRNAs to encode polypeptides[40]. Therefore, some DE lncRNAs can be involved in GTN tolerance through translated products, which will be investigated in the future.

5. Conclusions

Collectively, hundreds of mRNAs and lncRNAs were dysregulated in response to GTN tolerance. These findings might improve our understanding of the long-term GTN therapy and allow the pharmacological design of a suitable drug to prevent tolerance development. Further research is warranted to confirm our results in patients and to elucidate the exact role of the DE genes in GTN tolerance.

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硝酸甘油耐受大鼠主动脉差异表达基因与长链非编码RNA的鉴定与分析

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摘要: 硝酸甘油耐受极大地限制了其临床长期使用, 其发生机制尚未完全阐明。本研究分析了硝酸甘油耐受大鼠主动脉转录组的变化。通过RNA测序鉴定及检测mRNAs和lncRNAs, 利用IPA进行差异基因的通路分析, 使用RT-qPCR验证测序结果。在硝酸甘油耐受大鼠主动脉组织中, 我们共鉴定了22 788个基因(RPKM > 0.1, 其中有14 720个蛋白编码基因、4408个lncRNAs), 差异表达的mRNAs有115个(上调65个, 下调50个), 差异表达的lncRNAs有104个(上调56个, 下调48个)。IPA分析显示经典通路“Rho家族GTP酶信号通路”被抑制, 6个上游调控因子发生了改变, 11个基因与“血压异常”有关。通过对其邻近基因的分析来预测差异表达lncRNAs的顺式调控靶基因, 结果显示26个蛋白编码基因附近有28个差异表达lncRNAs, 许多差异表达mRNAs和差异表达lncRNAs的顺式调控靶基因与血压或细胞收缩的调节有关。本研究表明, 失调的mRNAs和lncRNAs参与了硝酸甘油耐受性的发生, 有望从中发现预防和逆转硝酸甘油耐受的潜在新靶点。

关键词: 转录组; 硝酸甘油; 耐受性; 长链非编码RNA; 主动脉