Characterization of IncRNA and mRNA profiles in rats with diabetic macroangiopathy

Chan Yang1*, Ziyan Xie2*, Qiangfei Yang3, Min Su2, Ran Yan2, Xueqin Cai2, Xiaoxu Fu2, Hong Gao1, Lian Du4, Wen Zhong1, Chunguang Xie1*

1 Hospital of Chengdu, University of Traditional Chinese Medicine, Chengdu, China, 2 Chengdu University of Traditional Chinese Medicine Clinical Medical College, Chengdu, China, 3 Department of Orthopaedics, The People’s Hospital of Jianyang city, Chengdu, China, 4 Chengdu University of Traditional Chinese Medicine, Chengdu, China

☯ These authors contributed equally to this work.
* xcg718@aliyun.com

Abstract

Diabetic macroangiopathy is part of the most common serious complications of diabetes. Previous studies indicate that IncRNAs involved in the process of diabetes and another vascular disease. However, their detailed mechanism of the IncRNAs involved in diabetic macroangiopathy has not been well characterized. In the present study, we generated rat models of diabetic macroangiopathy induced by High fat of 16weeks. A total of 15 GK rats were constructed as a test group, along with 15 Wistar rats set as control group, and thoracic aorta tissue from each group was collected. Whole genomic RNA sequencing was performed on thoracic aorta tissue; 3223 novel lncRNAs and 20367 annotated lncRNAs were indemnified in thoracic aorta samples, and 864 lncRNAs were expressed differently in the test and control groups. Gene ontology term enrichment showed the apparent enrichment of inflammatory response and cell apoptosis, which consistent with the results of H&E Staining, TUNEL Assay, and ELISA; Extensive literature reveals inflammatory response and cell apoptosis play an important role in the process of diabetic macroangiopathy. The results of the present study indicated that lncRNAs, especially Nrep. bSep08, Col5a1, aSep0, soygee.aSep08-unspliced, NONRATT013247.2, votar.aSep08-unspliced, etc, both participate in and mediate the process of inflammatory response, cell apoptosis. What’s more. Our research provides further insights into understanding of the basic molecular mechanisms underlying diabetic macroangiopathy.

Introduction

Diabetic macroangiopathy is currently the disease with the highest rate of death and disability among the complications of diabetes [1]. The present study showed that disorders of glucose and lipid metabolism, inflammation, oxidative stress, insulin resistance, and apoptosis are closely related to the occurrence and development of diabetic macroangiopathy [2–5]. With the completion of the Human Genome Project, more and more studies have shown that long non-coding RNA (IncRNA) can participate in the regulation of gene expression at the level of
epigenetic modification, transcription, and post-transcription [6]. Diabetes and its vascular
disease are closely related with lncRNA [7]; However, their detailed mechanism of the
lncRNAs involved in diabetic macroangiopathy was still in the enlightenment stage.

All experiments met all relevant regulatory standards. The male special pathogen-free (SPF)
Wistar rats (total 15) and Goto-Kakizaki (GK) rats (total 15) from Chinese Academy of Sci-
ences Shanghai Slack (SLAC) Laboratory Animals (Shanghai, China) weighing from 200 to
240g, were housed in an experimental animal center with an SPF-class experimental barrier
system of Sichuan Academy of Traditional Chinese Medicine (SYXK (Chuan) 2018–100). The
indoor temperature was maintained at 22 to 23 °C and the humidity was maintained at 55%
to 61%. The breeding method we adopted is divided into cages, 5 per cage, 6 cages in total. and
the cages were sterilized with 20 pounds autoclave for 15 minutes daily. Besides, The indoor
hygiene cleaning, cage changing/washing, etc, were all performed by professionally trained
personnel. We turned on indoor fluorescent lights to simulate daylight from 8:00 to 20:00 and
turned off the lights for the rest of the night to simulate night.

The GK rat model of diabetic macroangiopathy was generated through feeding with high
fat. the constituents of the high-fat diet include 88.2% of common animal feed add 10% refined
lard, 1.5% cholesterol, and 0.3% pig bile salt. While, the Wistar rats feeding in an ordinary diet,
respectively 16 weeks. Finally, there were anesthetized by 20% Ulatan (1g/kg i.p) after no diet
for 8 hours. We collected blood from the abdominal aorta which was centrifuged (3,000 rpm,
10 min) after being maintained at room temperature for 15 min. The serum was collected and
stored at –20˚C for ELISA. We peeled off the thoracic aorta immediately snap-frozen in liquid
nitrogen and stored at –80˚C for further experiments.

Materials and methods
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 protocol was
approved by the Committee on the Ethics of Affiliated Hospital of Chengdu University of Tra-
ditional Chinese Medicine. To ameliorate animals suffering, all related operations involving
animals during the entire experiment were carried out by professional and skilled technicians.
After the experiment, 20% Ulatan (1g/kg i.p) was used for rapid anesthesia.

Ulatran generally refers to Ethyl carbamate (C3H7NO2), 20% Uratan solution synthesis
from 20g Ulatan and 0.9% NS 100ml mixed. The reason why we choose uratan as an anesthetic
is that uratan has little effect on breathing.

Animal model of diabetic macroangiopathy

Determination of FPG, H&E staining, TUNEL assay for apoptosis, and determination
of IFN-γ, IL-4 in sera samples by ELISA. Every Saturday from 9 to 10 in the morning, the
blood glucose meter tail vein blood sampling was utilized to test the fasting blood glucose of
each group of rats. Before testing, fasting and water were not allowed for 8 hours. Blood was
collected with a sterile disposable needle tail vein. The blood glucose meter quickly draws
enough tail vein blood. After the value is shown, the blood glucose value is read and recorded
truthfully.

The thoracic aorta was fixed in 10% formaldehyde for 48 h. The specimens were subjected
to dehydration and permeabilization and then embedded in paraffin. The paraffin tissues were
sliced into serial sections (0.4 mm) and stained with H&E. Pathological changes were observed
under a microscope.

We used the TUNEL technique to detect apoptosis of thoracic aorta tissues. TUNEL apo-
ptosis used the DeadEndTM Fluorescence TUNEL System (Promega, G3250), and was
purchased from Promega Biotechnology (Beijing, China), and we performed the assay by the manufacturer’s instructions.

We detected IFN-γ, IL-4 in blood serum according to the instructions of the ELISA kit (Xitang, Shanghai, China). The concentration of each sample was calculated according to the optical density value and the standard curve.

**RNA-Seq and bioinformatics analyses.** Sequencing data were deposited with the NCBI. Total RNAs from rats subjected to experiment (n = 4) and controls (n = 4) were isolated and quality controlled. The preparation of whole-transcriptome libraries and next-generation sequencing were conducted by CHENGDU JUJIN BIOTECHNOLOGY CORPORATION (Chengdu, China). RNA-seq was performed on an Illumina Hiseq 4000 platform, and 150-bp-paired single-end reads were generated according to Illumina’s protocol. The procedures see Fig 1.

**The constructed of mRNA—lncRNA networks.** The differential lncRNA and differential mRNA co-expression was detected according to the Pearson correlation screen. The screening criteria were correlation coefficient coefficients > 0.7, P ≤ 0.05; Cytoscape software was used to generate the final differential lncRNA-differential mRNA co-expression network map.

**Statistical analysis**

We analyzed the data via software of SPSS16.0 and the data were presented as the mean ± SEM. one-way analysis of variance was used, and the LSD test was used for pairwise comparison when the data conform to normal distribution and homogeneity of variance, otherwise, Using non-parametric tests (rank-sum test). If there is no special explanation, P < 0.05 indicates statistical significance, that is, it was considered statistically significant.

**Results**

**Model identification of diabetic macroangiopathy in GK Rat with high fat feeding**

Our previous study shows that high-fat feeding can successfully induce macrovascular disease in GK rats. Thus, we generated a rat model of diabetic macroangiopathy induced via feeding
of high fat, 16 weeks. A total of 15 GK rats model was constructed and thoracic aorta from each was collected. The glucose levels >16.7 mmol/L was confirmed diabetic rat. The fasting plasma glucose (FPG) levels of each group list in Table 1. And the weight of rats in each group list in Table 2.

In the control group, H&E staining showed that the inner cell membrane remains complete, and there is no swelling of endothelial cells, the arrangement of cells is normal (Fig 2A). While in the test group, H&E staining showed that the inner cell membrane was impaired, the endothelial cells swelled and shed, and there are disordered cells and the reduction of the cell middle membrane and the uneven thickness of the vascular wall (Fig 2B); Besides, The thickness of thoracic aorta was measured, and we found that the thickness of thoracic aorta was significantly decreased in the GK rats of diabetic macroangiopathy (Table 3, Fig 2C). Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining of thoracic aorta showed a statistically significant increase in apoptosis rates in GK rats (Fig 2D–2F).

Besides, we examined serum inflammatory biomarkers (Interferon-γ (IFN-γ) and interleukin-4 (IL-4), simultaneously calculated the ratio of IFN-γ/IL-4 (Fig 2F–2H); the results showed that the level of the IL-4 (Fig 2H) were significantly reduced and the ratio of IFN-γ/IL-4 (Fig 2I) were significantly raised in the GK rats of diabetic macroangiopathy. Regrettably, the content of IFN-γ was no significantly raised in the test group (Fig 2G), we speculated that it may be related to the experiment time, no matter what, it still keeps rising.

RNA sequencing identified the features of IncRNA and mRNA in diabetic macroangiopathy of GK rats

We analyzed RNA sequencing (RNA-seq) data from 4 rats randomly selected from each group thoracic aorta samples, in which 61,000,000–82,000,000 raw data and 57,000,000–79,000,000 clean data were obtained (Table 4). The classification of mapped reads in each thoracic aorta is shown in Table 5. The Fluorometer Quantitative Results (Table 6), Q value box chart (Fig 3A and 3B), and Base distribution diagram (Fig 3C and 3D) indicated all samples were sequenced per standards and data analysis was possible.

Differential expression and cluster analysis of mRNAs and IncRNAs

We analyzed differential expression (DE) IncRNAs and mRNAs using significance analysis following the criterion \( q < 0.05 \) to prove whether IncRNAs were involved in the pathological process of diabetic macroangiopathy.

The results showed that there were DE 2165mRNAs (664 upregulated and 1501 downregulated) and 864 DE IncRNAs (550 upregulated and 314 downregulated) (Fig 4A and 4B). The expression pattern of DE mRNAs and IncRNAs is shown using a clustered heatmap (Fig 4C and 4D).

The detailed information of the top 10 upregulated and 10 downregulated IncRNAs and mRNA summarized in Tables 7 and 8. These results reflect distinct IncRNAs and mRNA

Table 1. FPG of rats in each group (X ± SEM, mmol/l), comparison of two groups.

| Group     | N | FPG       |
|-----------|---|-----------|
| Control   | 15| 6.53±0.17 |
| Model     | 15| 22.61±1.54*** |

\*\*\* \( p < 0.01 \).

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expression profiles between the test group and the control group, implying different underlying pathophysiology in diabetic macroangiopathy.

**Systematic functional analysis of differentially expressed lncRNAs and mRNAs.** To clarify the possible functional significance of observed changes in lncRNAs and mRNAs levels between the test group and the control group, we performed a Gene Ontology (GO) term enrichment analysis. There were 5587 background genes in total. We summarized the significantly enriched GO terms of mRNAs (Fig 5A) and lncRNAs (Fig 5B) regarding the biological process, cellular component, and molecular function, respectively. Notably, we found that the DE of lncRNAs and mRNAs were similar and significantly related to inflammatory response and cell apoptosis in biological process term enrichment. Moreover, the GO term RNA regulation, metabolic processes, cell killing, cell aggregation, the cellular process, and immune systems was a significant enrichment on both the lncRNAs and mRNAs levels, which indicates that lncRNAs and mRNAs could make sense in the pathological process of diabetic macroangiopathy, and inflammatory response and cell apoptosis might play a key role in diabetic macroangiopathy. Apart from this, the enrichment of cellular components and molecular function also showed a similar pattern. Such as, cell junction, cell part, remembrance, and antioxidant activity were all enriched on the lncRNA and mRNA levels (Fig 5A and 5B), which revealed that complex pathological processes are involved in diabetic macroangiopathy.

To certificate if there were some specific pathways changed in diabetic macroangiopathy, we performed the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis in lncRNA target genes and mRNAs. It is noteworthy that the PPAR signaling pathway, fatty acid metabolism, and fatty acid degradation was also enriched in both lncRNAs and mRNAs (Fig 5C and 5D). The AMPK signaling pathway, JAK/STAT signaling pathway, Akt/mTOR pathway, Cellular Signaling Pathways were also enriched, which is consistent with previous research and consistent with the GO term enrichment results.

**Diabetic macroangiopathy-related differential lncRNA-mRNA co-expression network**

Pearson’s correlation coefficient (PCC) was computed between the expression values of each of the lncRNA-mRNA pairs across the two group’s samples (PCC>0.7, P<0.05), respectively. Subsequently, the lncRNA-mRNA co-expression network was constructed by the Cytoscape (Fig 6). The core lncRNAs of the top 10 shown as follow in Table 9.

**Discussion**

Diabetic macroangiopathy, as a common complication of diabetes, usually causes multi-organ dysfunction and can lead to a high risk of disability and mortality [8]. Diabetic macroangiopathy, like a low-grade inflammation (chronic inflammation) state [9], is a direct result of the chronic low-grade inflammatory response of the blood vessel wall to endothelial cells. Various inflammatory mediators such as tumor necrosis factor, CRP, and IFN-γ are closely related to
Fig 2. The basic characteristics between test group and the control group: (2A-2C): Pathological comparison in thoracic aorta tissue and vascular thickness through H&E staining (*×200), n = 6 in each group. (2Dand 2F): Apoptosis detection in horacic aorta tissue by TUNEL staining (200×), n = 6 in each group. (2G-2H) Serum inflammatory factor (IFN-γ and IL-4) were detected by ELISA; (2I): The ratio of IFN-γ/IL-4 were calculated via Spass, n = 15 in control and n = 14 in test group (1 GK rat died of ketosis caused by hyperglycemia); *p < 0.05 compared to the control; **p < 0.01 compared to the control.

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### Table 3. Thickness of thoracic aorta (um) (X ± S, um), comparison of two groups.

| Group | N  | 12W   |
|-------|----|-------|
| Control | 6  | 158.23±15.47 |
| Test   | 6  | 88.72±5.26   |

★★ P<0.01, ★ P<0.05.

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### Table 4. The detailed information of RNA sequencing.

| Sample ID | Raw reads | Clean reads | Clean ratio | rRNA trimmed | rRNA ratio | No rRNA pair |
|-----------|-----------|-------------|-------------|--------------|------------|--------------|
| Control 1 | 79,978,968 | 76,603,868  | 95.78%      | 76,431,593   | 0.22%      | 75,076,160   |
| Control 2 | 61,334,798 | 57,848,275  | 94.32%      | 57,704,683   | 0.25%      | 56,410,154   |
| Control 3 | 72,302,776 | 68,961,193  | 95.38%      | 68,866,487   | 0.14%      | 67,542,192   |
| Control 4 | 82,377,968 | 79,307,418  | 96.27%      | 79,242,358   | 0.08%      | 78,125,230   |
| Test 1   | 81,099,852 | 77,710,102  | 95.82%      | 77,510,349   | 0.26%      | 76,186,540   |
| Test 2   | 75,341,414 | 72,013,132  | 95.58%      | 71,959,269   | 0.07%      | 70,642,008   |
| Test 3   | 76,767,478 | 73,523,404  | 95.77%      | 73,449,426   | 0.10%      | 72,275,094   |
| Test 4   | 79,208,782 | 75,857,586  | 95.77%      | 75,689,620   | 0.22%      | 74,322,602   |

Clean ratio = (Clean reads/Raw reads) %; rRNA ratio = [(Clean reads—rRNA trimmed)/Clean reads] %.

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### Table 5. Results of mapping genome.

| Samples ID | All reads | Mapped reads | Mapped Pair Reads | Mapped broken-pair reads | Mapped Unique reads | Mapped Multi reads | Mapping ratio |
|------------|-----------|--------------|-------------------|--------------------------|---------------------|------------------|---------------|
| Control 1  | 75,076,160| 68,954,951   | 67,884,106        | 1,070,845                | 68,697,396          | 257,555          | 91.85%        |
| Control 2  | 56,410,154| 47,921,516   | 47,141,556        | 779,960                  | 47,710,257          | 211,259          | 84.95%        |
| Control 3  | 67,542,192| 62,147,744   | 61,191,752        | 955,992                  | 61,932,608          | 215,136          | 92.01%        |
| Control 4  | 78,125,230| 70,475,378   | 69,405,918        | 1,069,460                | 70,233,818          | 241,560          | 90.21%        |
| Test 1     | 76,186,540| 69,416,188   | 69,141,188        | 1,063,150                | 70,242,821          | 236,517          | 92.51%        |
| Test 2     | 70,642,008| 65,074,288   | 65,074,288        | 987,379                  | 65,848,086          | 213,581          | 93.52%        |
| Test 3     | 72,275,094| 66,276,442   | 66,276,442        | 1,054,271                | 67,103,601          | 227,112          | 93.16%        |
| Test 4     | 74,322,602| 67,082,953   | 67,082,953        | 1,011,963                | 66,865,658          | 217,295          | 90.26%        |

Mapping ratio = Mapped reads/All reads, Mapped Unique reads.

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### Table 6. Qubit® 2.0 fluorometer quantitative results.

| No | Sample Name | Index No. | Conc (ng/μL) | Peak Lenth (bp) |
|----|-------------|-----------|--------------|-----------------|
| 1  | Control 1   | V17       | 39.8         | 411             |
| 2  | Control 2   | V18       | 32.4         | 404             |
| 3  | Control 3   | V19       | 37.2         | 410             |
| 4  | Control 4   | V20       | 46.8         | 407             |
| 5  | Test 1      | V21       | 40.8         | 411             |
| 6  | Test 2      | V22       | 40.2         | 410             |
| 7  | Test 3      | V23       | 45.4         | 410             |
| 8  | Test 4      | V24       | 38.8         | 415             |

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the occurrence and development of diabetic macroangiopathy, Can be used as a predictor of diabetic macroangiopathy. Therefore, in this study, IL-4 and IFN-γ were selected as one of the markers for judging diabetic macrovascular injury. At the same time, the pathogenesis of diabetic macroangiopathy was discussed in depth from lncRNA.

To simulate the pathological process of diabetic macroangiopathy, we used a GK rat model fed with a high-fat of 16 weeks, which showed an obvious endothelial dysfunction (included endothelial injury and endothelial apoptosis). Many previous experiments have shown that the model is relatively stable and has a high recurrence rate. therefore, it is suitable for studying the mechanism of diabetic macroangiopathy [10].

At present, there are two types of gene sequencing including microarray sequencing and next-generation high-throughput sequencing. Compared with microarrays, next-generation sequencing has the advantages of high sensitivity, specificity, and the ability to discover important new lncRNAs. With the development of next-generation high-throughput sequencing technology, multiple lncRNAs have been found to play important roles in diabetes and its
complications [5]. However, no related studies have been found to use whole-genome sequencing to report and compare changes in lncRNAs and mRNAs in diabetic macroangiopathy.

To recognize the basic characteristics of lncRNAs and mRNAs and find the potential role of lncRNAs in diabetic macroangiopathy, our study evaluated entire lncRNAs and mRNAs changing significantly in GK rat thoracic aorta tissues compared with the control group. What’s more, to increase the reliability and validity of sequencing, we exploited 4 GK rats of diabetic macroangiopathy compared with 4 control rats to find the ubiquitous differential expression lncRNAs and mRNAs. Base on quality control, this sequencing results had high reliability and quality. We predicted potential functions of lncRNAs and mRNAs by GO term enrichment and KEGG pathway enrichment, via bioinformatics analysis, and the results were beneficial for us to study the molecular mechanisms of diabetic macroangiopathy more clearly. Our study indicates that lncRNAs, including Nrep.bSep08, Col5a1.aSep0, soogee.aSep08-unspliced, NONRATT013247.2, votar.aSep08-unspliced, etc play key roles in the pathological process of diabetic macroangiopathy and provide a new direction toward understanding this pathological process.
Table 7. The detailed information of the top 10 upregulated/downregulated mRNA.

| Gene id | Gene name | Locus | log2fc | p       | up/down |
|---------|-----------|-------|--------|---------|---------|
| 37436   | Macc1     | 6     | inf    | 6.34E-08| down    |
| 03136   | Fcrla     | 13    | inf    | 6.56E-08| down    |
| 24221   | Mettl24   | 20    | inf    | 1.92E-07| down    |
| 57008   | AABR07010985.1 | 2 | inf    | 1.87E-06| down    |
| 28576   | Calml5    | 17    | inf    | 2.76E-06| down    |
| 04341   | LOC498222 | 13    | inf    | 1.12E-05| down    |
| 29262   | RGD1560017| 3     | inf    | 3.89E-05| down    |
| 55434   | AABR07026641.1 | 16 | inf    | 5.62E-05| down    |
| 20873   | Nphs1     | 1     | inf    | 0.000114| down    |
| 42468   | AABR07066754.1 | 9 | inf    | 0.000138| down    |
| 20985   | Atp4a     | 1     | inf    | 1.62E-17| up      |
| 13128   | Tmem179   | 6     | inf    | 1.21E-13| up      |
| 03284   | Epn3      | 10    | inf    | 2.20E-13| up      |
| 60029   | AABR07072539.4 | 17 | inf    | 1.08E-08| up      |
| 03273   | Spata20   | 10    | inf    | 3.39E-07| up      |
| 48522   | LOC100909648 | 13 | inf    | 9.92E-07| up      |
| 46250   | LOC100911027 | 1  | inf    | 4.98E-06| up      |
| 33983   | Solh1     | 3     | inf    | 1.85E-05| up      |
| 53562   | Arx       | X     | inf    | 3.95E-05| up      |
| 47520   | Rn60_5_0815.11 | 5  | inf    | 5.94E-05| up      |

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Table 8. The detailed information of the top 10 upregulated/downregulated IncRNAs.

| IncRNA_id | IncRNA_name | length | locus | log2fc | P       | up/down |
|-----------|-------------|--------|-------|--------|---------|---------|
| NONRATT008127.2 | RGD1559582.aSep08-unspliced | 274    | 13    | inf    | 8.37E-14| down    |
| NONRATT026294.2 | NONRATT026294.2 | 862    | 7     | inf    | 3.69E-11| down    |
| NONRATT007737.2 | Rpl21.eSep08 | 601    | 12    | inf    | 4.41E-10| down    |
| NONRATT015712.2 | NONRATT015712.2 | 2766   | 2     | inf    | 1.01E-07| down    |
| NONRATT023839.2 | forwee.aSep08-unspliced | 761    | 5     | inf    | 3.93E-07| down    |
| NONRATT016766.2 | Gstm2.bSep08 | 402    | 2     | inf    | 9.24E-06| down    |
| NONRATT029734.2 | LOC689856.aSep08-unspliced | 538    | 9     | inf    | 3.07E-05| down    |
| NONRATT028171.2 | Mobp.cSep08 | 791    | 8     | inf    | 0.0001 | down    |
| NONRATT011382.2 | Gipbp3.cSep08 | 743    | 16    | inf    | 0.0001 | down    |
| ENSRNOT0000078751 | ENSRNOT0000078751 | 4140   | 2     | inf    | 0.0002 | down    |
| NONRATT020147.2 | NONRATT020147.2 | 611    | 4     | inf    | 1.57E-26| up      |
| NONRATT001894.2 | tolo.aSep08 | 842    | 1     | inf    | 2.00E-13| up      |
| ENSRNOT0000085586 | ENSRNOT0000085586 | 2426   | 17    | inf    | 1.63E-06| up      |
| NONRATT002575.2 | Efrb.cSep08 | 614    | 1     | inf    | 1.67E-06| up      |
| NONRATT023574.2 | Cap1.hSep08 | 552    | 5     | inf    | 3.70E-06| up      |
| NONRATT031178.2 | NONRATT031178.2 | 622    | X     | inf    | 5.14E-05| up      |
| NONRATT022004.2 | korbo.aSep08-unspliced | 325    | 5     | inf    | 9.51E-05| up      |
| NONRATT011770.2 | peegar.aSep08-unspliced | 1231   | 16    | inf    | 0.0002 | up      |
| NONRATT025413.2 | V-set.38.aSep08 | 411    | 6     | inf    | 0.0006 | up      |
| NONRATT011006.2 | speejar.aSep08-unspliced | 911    | 14    | inf    | 0.0014 | up      |

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We identified 864 DE lncRNAs between test group and control groups; the potential mechanisms of these lncRNAs are still unclear. We also conducted GO enrichment and found that the terms of biological processes occupied a great majority of the significantly enriched terms. Based on the KEGG enrichment results, we found that the PPAR signaling pathway, fatty acid metabolism, and fatty acid degradation were the most common pathways that emerged at both the lncRNA and mRNA levels. Even though some researches have shown the probable role of the aforementioned common pathways in some metabolic diseases [11–13], the regulatory relationship between lncRNAs and these pathways is still poorly understood, and more in-depth research is needed in the future basic studies.

With the introduction of the “Inflammatory Response Theory” by Professor Ross in 1993 [14], More and more studies have shown that diabetic macroangiopathy is an inflammatory...
It is believed that vascular endothelial injury is the initiating factor of diabetic macroangiopathy \cite{16}. And is a direct result of the chronic low-grade inflammatory response of endothelial cells. Various inflammatory mediators such as IFN-γ and IL-4 are closely related to the occurrence and development of diabetic macroangiopathy \cite{17}. Cell apoptosis, the pathological basis of endothelial dysfunction, will lead to the imbalance of the body’s homeostasis, and plays an important role in diabetic macroangiopathy \cite{18}. We detected a significant difference in both cell apoptosis and the levels of the inflammatory factor, including IFN-γ, IL-4, and IFN-γ/IL-4 in thoracic aorta tissue from the test and control groups. These results are also matching with the results of the GO term and KEGG pathway enrichment.
Our study is to explore the role of LncRNAs in the pathological process of diabetic macroangiopathy. By applying whole-genome next-generation sequencing, we compared the differences of LncRNAs and mRNAs between test and control groups; this research indicates that LncRNAs play a critical role in the pathogenesis of diabetic macroangiopathy. and this provided a new perspective for the study of the pathological mechanism of diabetic macroangiopathy, and also provided new ideas for the future diagnosis and treatment of diabetic macroangiopathy.

Problems and Prospects: This experiment explored the mechanism of mRNA/LncRNA expression profile differences, and also screened out related differential genes, but no relevant verification was performed. The next step could be PCR quantification, WB protein qualitative and quantitative verification, and methyl In chemical verification, primary cell culture, siRNA interference, and gene knockout can also be used to verify related core LncRNA.

Author Contributions

Conceptualization: Chan Yang, Ziyan Xie.

Data curation: Chan Yang, Xueqin Cai.

Formal analysis: Chan Yang.

Investigation: Chan Yang, Chunguang Xie.

Methodology: Chan Yang, Min Su, Ran Yan, Xiaoxu Fu, Hong Gao, Chunguang Xie.

Project administration: Chan Yang.

Resources: Chan Yang, Qiangfei Yang.

Software: Qiangfei Yang.

Supervision: Lian Du, Chunguang Xie.

Validation: Lian Du.

Visualization: Wen Zhong.

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