Abnormal Peripheral Neutrophil Transcriptome in Newly Diagnosed Type 2 Diabetes Patients

Application of the MGI DNBSEQ Sequencing Platform in the evaluation of differentially expressed genes (DEGs) in Diabetes

A research team at The Second Xiangya Hospital of Central South University in China analyzed the differentially expressed genes (DEGs) of neutrophils in the blood of patients with type 2 diabetes versus a control group using the MGI DNBSEQ sequencing platform. Their results illustrate the relationship between neutrophils and human type 2 diabetes mellitus.

The relevant results of this study were published in 2020 in the *Journal of Diabetes Research*, under the title "Abnormal Peripheral Neutrophil Transcriptome in Newly Diagnosed Type 2 Diabetes Patients"[1].

Recommended application: Disease Omics (Chronic Diseases)

Recommended model: DNBSEQ-G400RS

- ### Fitting equipment for automated library preparation

  Automated library construction platforms such as the MGISP-100 and the MGISP-960 ensure efficient and accurate library construction for high-throughput workflows on the DNBSEQ-G400RS sequencer.

Background

Diabetes mellitus (DM) refers to a metabolic disorder caused by either defective insulin secretion, inappropriate insulin action, or a combination of both. A rapid increase in DM prevalence and number of patients poses a great challenge to the global health system. As of 2021, approximately 537 million adults worldwide had been diagnosed with DM and that number is expected to be 783 million in 2045[2,3]. DM is mainly classified into type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), with T2DM accounting for nearly 95% of cases. T1DM is an autoimmune disease featuring insufficient insulin secretion due to T cell-mediated destruction of insulin-secreting pancreatic beta cells[4]. T2DM involves the progressive development of insulin resistance (IR) in the liver and peripheral tissues in addition to reduced number of β-cells and insufficient insulin secretion. The disease is characterized by disorders of glucose, lipid, and protein metabolism, and IR is associated with low-grade chronic inflammation[5,6]. T2DM can damage multiple organs and lead to various complications. Diabetic nephropathy, diabetic foot, diabetic neuropathy, and cardiovascular diseases associated with T2DM have become major global health problems[7].

As important components of the innate immune system, neutrophils have been found to participate in tissue repair, for they can engulf and/or release neutrophil extracellular trap (NET) to fight against the pathogens[8]. Neutrophil-directed chronic inflammation can be achieved by inhibiting the release of NETs or decomposing NETs. Additionally, neutrophil-derived particles can lead to an anti-inflammatory response, which is an innovative strategy to reduce inflammation[9]. Given the important role of chronic inflammation in the occurrence and development of DM, as well as the pathogenesis of its complications, and the implication of neutrophils in the inflammatory response[10], the neutrophil/lymphocyte ratio (NLR) has recently been adopted as a new inflammatory biomarker of DM and is important for pathological diagnosis[11].

Study description

The exact mechanism of neutrophil-induced T2DM has not been clarified. RNA-seq analyzes changes in gene expression across the transcriptome. Sensitive and useful in identifying novel transcripts, RNA-seq has been applied in an increasing number of research studies. In this study, the research team studied the transcriptomic changes of neutrophils in circulating blood of T2DM patients using RNA sequencing to elucidate the response mechanism of neutrophils in T2DM.

Materials and Methods

Sample collection and RNA preparation

According to the diabetes prevalence criteria determined by the World Health Organization in 1999, the team recruited 13 patients from The Second Xiangya Hospital of Central South University who had been diagnosed within less than one year with T2DM. They also recruited healthy volunteers of similar gender and age as controls. All patients and members of the control group were divided into the discovery group (n=5:5) and validation group (n=8:8). The team isolated neutrophils from venous blood of patients and controls using density-gradient centrifugation. After additional purification, the neutrophils were resuspended in TRIzol (5-10×106cells/1ml) and stored at -80℃ for subsequent experiments. For those experiments, total RNA was extracted, the concentration was determined, and the RNA was reverse transcribed into cDNA with a cDNA reverse transcription kit.

RNA sequencing and bioinformatic analysis

After the construction of RNA-Seq libraries, samples were sequenced on the MGI DNBSEQ sequencing platform. A total of 19,718 genes were detected, and the average data volume for each sample was 24.04M reads.

In the subsequent bioinformatic analysis, the team first performed quality control screening and filtering of all data. They then compared clean reads to the reference genome using HISAT and Bowtie2 and determined gene expression with the software package RESM.

Based on gene expression levels, the research team screened DEGs in T2DM patients and the control group via a DEG-seq algorithm. The identified DEGs were then classified according to molecular biological function, cellular components, and biological process using Gene Ontology (GO). Subsequently, the team carried out a KEGG pathway classification and functional enrichment of the DEGs with phyper in R.

Sample collection and preparation	Library preparation and sequencing	Bioinformatics Analysis	Result analysis
13 type 2 diabetes patients (diagnosed within less than 1 year) and 13 patients of similar gender and age for the control group	 MGIEasy RNA Library Preparation Kit  DNBSEQ-G400RS Genetic Sequencer	HISAT Bowtie2 RESM DEG-seq Algorithm KEGG	Gene Ontology (GO) Classification, KEGG Pathway Enrichment Analysis

Result analysis

Participant characteristics

The team divided the 26 participants (T2DM patients and control volunteers) into a discovery group and a validation group to analyze anthropometric characteristics. The results showed that compared to controls, T2DM patients exhibited higher values in HbA1c percentage, postprandial blood glucose levels, fasting c-peptide concentration, and blood pressure ($P < 0.05$). There were no significant differences in BMI, HER, fasting blood glucose, LDL-C and TC (Table 1). In the validation group, HbA1c and postprandial blood glucose levels were higher in T2DM patients compared to the control group (Table 2).

	HC ($n = 5$)	T2D ($n = 5$)	*P* value
Sex (male/female)	5 (4/1)	5 (3/2)	1.000
Age (years)	43.40 ± 13.22	41.40 ± 7.50	0.776
BMI (kg/m^2)	23.18 ± 2.21	23.63 ± 13.27	0.942
WHR	0.86 ± 0.06	0.92 ± 0.03	0.081
DBP (mmHg)	74.40 ± 5.32	81.80 ± 5.85*	0.028
SBP (mmHg)	106.20 ± 8.95	124.00 ± 11.85*	0.029
TG (mmol/L)	1.03 ± 0.56	1.86 ± 0.62	0.056
TC (mmol/L)	4.22 ± 0.49	5.15 ± 1.27	0.166
HDL-C (mmol/L)	1.47 ± 0.44	1.17 ± 0.14	0.210
LDL-C (mmol/L)	2.34 ± 0.54	3.42 ± 1.23	0.109
HbA1c (%)	5.52 ± 0.46	7.80 ± 2.04*	0.040
Fasting BS (mmol/L)[a]	5.16 (4.88~5.36)	9.88 (5.52~10.22)	0.082
2 h postprandial BS (mmol/L)	4.88 ± 1.64	12.58 ± 5.17*	0.013
Fasting C-peptide (pmol/L)	350.36 ± 90.08	707.36±207.37**	0.008
2 h postprandial C-peptide (pmol/L)	1535.70 ± 549.43	1209.26 ± 82.11	0.225
White cell count (10^9/L)	6.38 ± 1.35	7.70 ± 1.92	0.241
Lymphoid cell count (10^9/L)	1.80 ± 0.47	2.61 ± 0.64	0.052
Neutrophil count (10^9/L)	4.15 ± 1.25	4.68 ± 1.59	0.569
Mononuclear count (10^9/L)	0.32 ± 0.12	0.31 ± 0.09	0.822

Table 1. Clinical and biochemical characteristics of RNA-seq study participants.

	HC ($n = 8$)	T2D ($n = 8$)	*P* value
Sex (male/female)	8 (6/2)	8 (6/2)	1.000
Age (years)	44.25 ± 8.41	47.63 ± 10.74	0.496
BMI (kg/m^2)	22.40 ± 2.10	23.82 ± 2.83	0.273
WHR	0.85 ± 0.08	0.88 ± 0.05	0.388
DBP (mmHg)	75.85 ± 7.85	79.88 ± 8.01	0.316
SBP (mmHg)	121.00 ± 11.20	118.50 ± 16.45	0.733
TG (mmol/L)	1.61 ± 0.81	1.54 ± 1.58	0.903
TC (mmol/L)[a]	4.77 (4.05~4.93)	3.96 (2.50~4.56)	0.050
HDL-C (mmol/L)	1.14 ± 0.48	1.51 ± 0.92	0.332
LDL-C (mmol/L)	2.75 ± 0.36	2.07 ± 0.89	0.065
HbA1c (%)[a]	5.30 (5.20~5.55)	6.50 (6.05~7.70)***	<0.001
Fasting BS (mmol/L)[a]	4.61 (4.21~5.11)	5.97 (5.14~7.65)**	0.002
2 h postprandial BS (mmol/L)	4.88 ± 1.64	12.58±5.17***	<0.001
Fasting C-peptide (pmol/L)	418.71 ± 90.49	369.44 ± 141.28	0.420
2 h postprandial C-peptide (pmol/L)	1598.80 ± 711.38	1229.03 ± 548.98	0.264
White cell count (10^9/L)	5.97 ± 0.81	5.73 ± 0.42	0.411
Lymphoid cell count (10^9/L)	1.97 ± 0.54	1.83 ± 0.83	0.695
Neutrophil count (10^9/L)[a]	3.41 (3.12~3.50)	3.42 (2.88~3.68)	0.878
Mononuclear count (10^9/L)	0.40 ± 0.08	0.30 ± 0.07	0.095

BMI: Body mass index; WHR: Waist-hip ratio; DBP: Diastolic blood pressure; SBP: Systolic blood pressure; TG: Triglyceride; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol.

Table 2. Clinical and biochemical characteristics of RNA-seq study participants.

Bioinformatic analysis

The research team sequenced five samples from T2DM patients and five samples from the control group on the DNBSEQ sequencing platform. They performed an RNA sequence analysis of neutrophils and identified DEGs. The results are shown in Figures 1 and 2. Compared with neutrophils of the control group, 1,990 DEGs were upregulated and 1,314 DEGs were downregulated in neutrophils of T2DM patients.

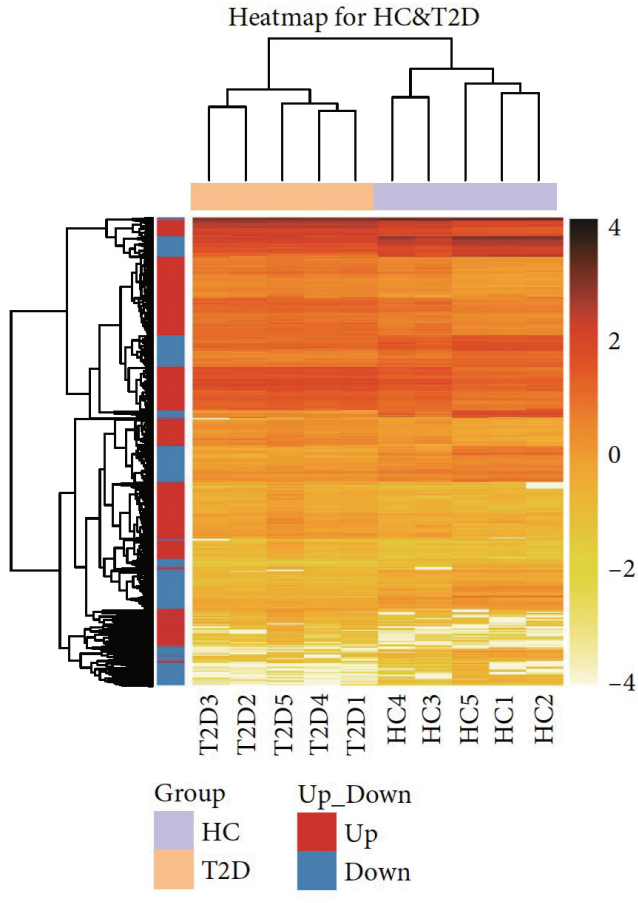

Fig. 1. Peripheral neutrophil transcriptome of newly diagnosed T2DM (T2D) patients differs from that of control samples. The heatmap shows neutrophil DEGs isolated from the control group (purple, n = 5) and newly diagnosed T2D patients (orange, n = 5).

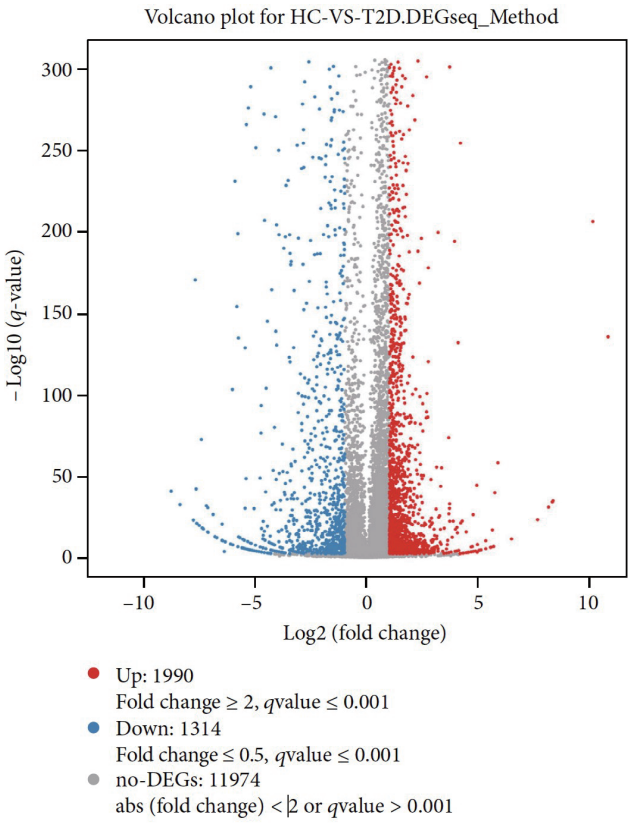

Fig. 2. Volcano map shows the number of DEGs in peripheral blood neutrophils of newly diagnosed T2DM patients and controls.

Gene Ontology (GO) Classification

The study team classified the DEGs according to GO to determine the molecular functions (MM), cellular components (CC), and biological processes (BP) in which the proteins encoded by these genes were involved. The upregulated GO categories were found to be regulatory pathways of myeloid leukocytes, T cells and immune response, while the downregulated GO categories were mainly bacteria-derived molecular responses and inflammatory responses (Fig. 3).

KEGG Pathway Enrichment Analysis

KEGG pathway enrichment analysis of important DEGs showed that multiple biological

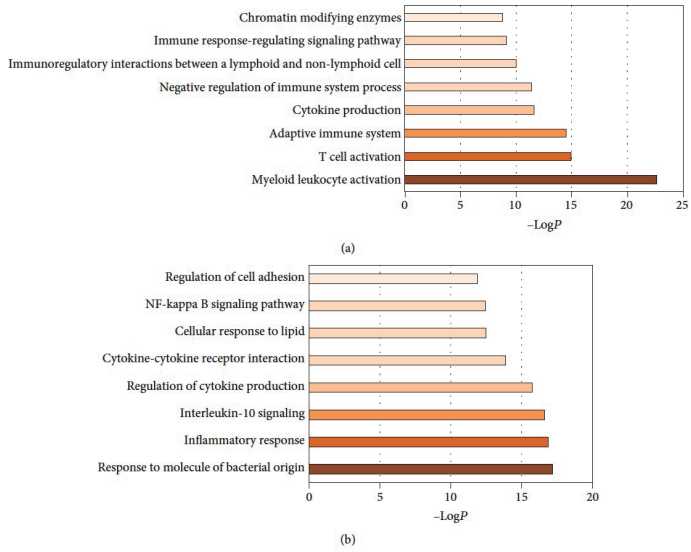

Fig. 3. GO analysis of enrichment of DEGs between T2DM patients and controls. (a) Top 8 classes of GO upregulated genes; (b) top 8 classes of GO downregulated genes.

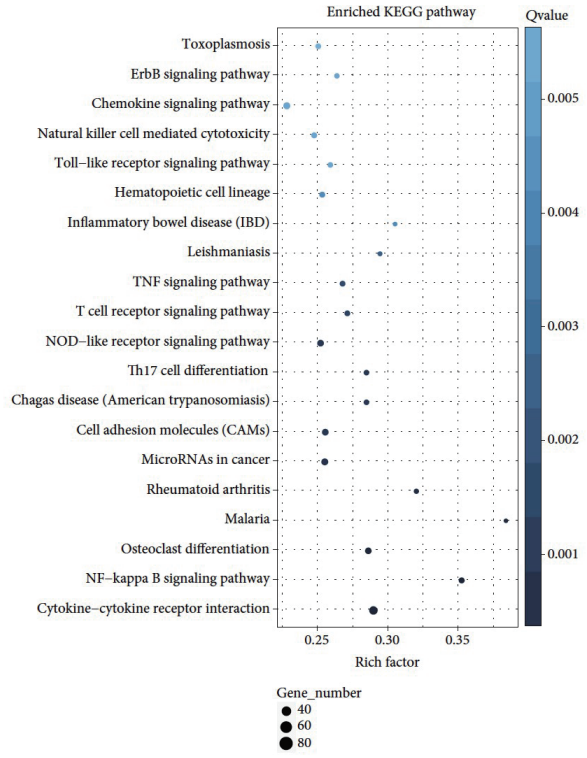

Fig. 4. Top 20 KEGG pathways screened based on all DEGs detected in neutrophils of T2DM patients and controls.

pathways changed in neutrophils of T2DM patients compared to the control group (Fig. 4). The relevant differential pathways involved mainly the cytokine-cytokine receptor interaction and signaling such as NF-κB. Most of the detected genes associated with cytokine-cytokine receptor interaction and cell adhesion

Real-time quantitative PCR

As a next step, the team expanded the analysis to purified neutrophils. RNA-seq results showed that genes were directly associated with neutrophil activation, such as the expression of adhesion molecules and related ligands or receptors, and increased expression of CXCR1, CXCR2, and other genes (Fig. 6).

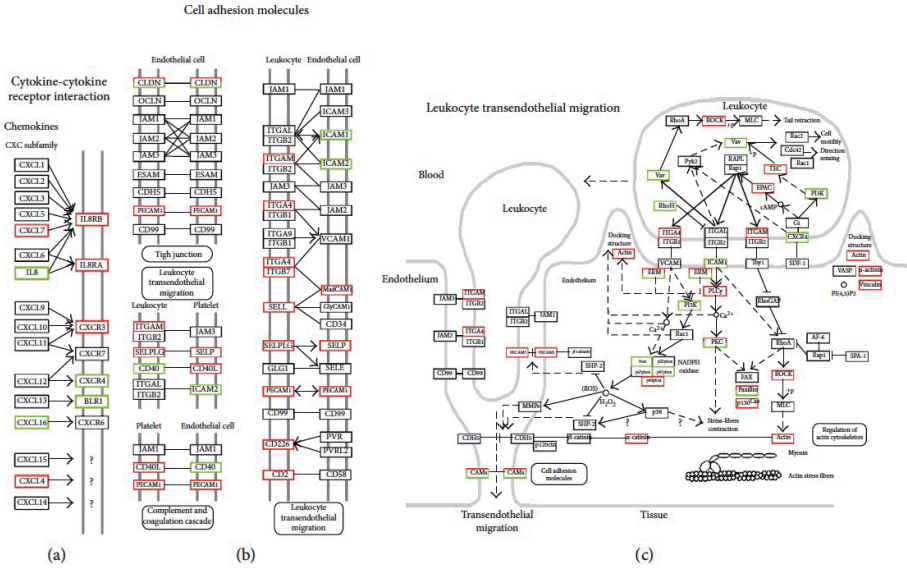

Fig. 5. Enrichment pathway of DEGs in neutrophils of T2DM patients compared to controls.

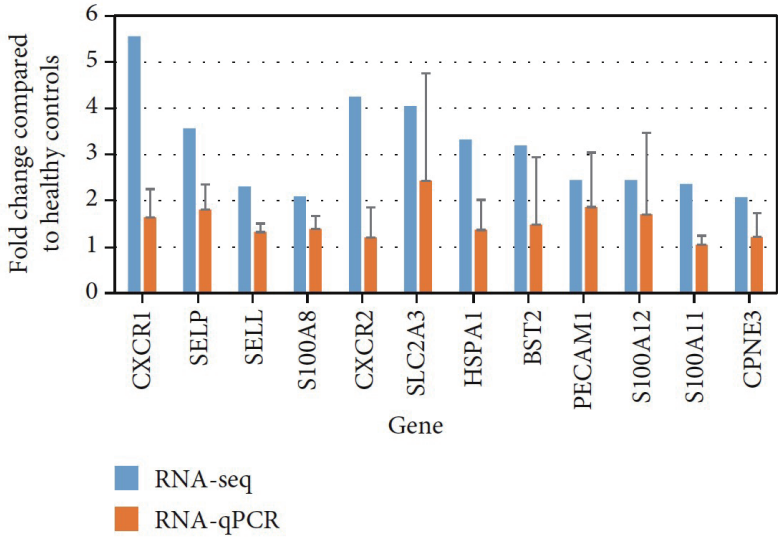

Fig. 6. RT-qPCR validation of RNA-seq results from 12 subsets of genes.

Conclusion

This study investigated the presence of DEGs in neutrophils of T2DM patients compared to controls, and the biological functions associated with these genes. The results showed that T2DM patients had increased neutrophil activation at the transcriptome level, increased response to chemokines, and increased neutrophil migration across endothelial cells. furthermore, the T2DM patients showed decreased neutrophil response to bacteria-derived molecules (e.g., LPS), cellular response to bacteria, and inflammatory response. These findings supported the role of neutrophils in the pathogenesis of T2DM.

For this study, the team compared DEGs between healthy subjects and T2DM patients via RNA-seq using the DNBSEQ sequencing platform independently developed by MGI. The study results played an important role in determining the underlying pathological relationship between T2DM and neutrophils, potentially providing new targets for the prevention and treatment of DM[12]. Hallmarks of the DNBSEQ sequencing technology are high accuracy, fidelity, signal- to-noise ratio, and cost effectiveness, making it a widely used tool in scientific research and clinical studies.

DNBSEQ-G400RS Genetic Sequencer

Reference

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Recommended Ordering Information

Category	Product	Cat. NO.
Instruments	DNBSEQ-G400RS Genetic Sequencer	900-000170-00
	MGISP-100RS Automated Sample Preparation System	900-000206-00
	MGISP-960RS Automated Sample Preparation System	900-000146-00
Software	MegaBOLT Bioinformatics analysis accelerator	900-000555-00
Library Prep	MGIEasy RNA Library Prep Set（16 RXN）	1000006383
Sequencing Reagents	DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE100)	1000016950

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