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Identification of hub genes and pathways in mouse with cold exposure

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Abstract

Background: Cold exposure is linked to numerous diseases, yet the changes in key genes and pathways in mice under cold exposure remain unexplored. Understanding these alterations could offer insights into the mechanisms of cold resistance and contribute valuable ideas for treating cold-related diseases. **Methods:** The dataset GSE148361 was obtained from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) were identified using the "limma" package in R software. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed on DEGs. The STRING (Search Tool for the Retrieval of Interacting Genes) database was used to construct a protein-protein interaction (PPI) network. Additionally, gene set enrichment analysis (GSEA) was conducted to identify pathways associated with key genes. miRNAs and upstream transcription factors (TFs) were predicted using the miRNet database. **Results:** A total of 208 DEGs were identified, with 137 upregulated and 71 downregulated. In biological processes, DEGs were enriched in nucleotide and purine-containing compound metabolism. For cellular components, DEGs were involved in condensed chromosomes and mitochondrial protein complexes. In molecular functions, proton transmembrane transporter activity was enriched. KEGG pathway analysis showed significant enrichment in biosynthesis of unsaturated fatty acids, fatty acids, and pyruvate metabolism. From the PPI network, 12 hub genes were identified using MCODE. Four hub genes (*Col3a1, Ifi203, Rtp4, Vcan*) demonstrated similar trends in a validation set (GSE110420) and were significantly differentially expressed. GSEA analysis indicated that these four genes were enriched in pathways such as ECM-receptor interaction and cytokine-cytokine receptor interaction. The hub gene network included 93 miRNAs and one TF. **Conclusion:** This study identified four hub genes as potential diagnostic biomarkers for cold exposure, providing insights for further research on the effects of cold on gene expression and disease.

Keywords

cold exposure; hub genes; pathway; adipose tissue

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1 Introduction

Current research suggests that humans experience cold exposure in environments below 20°C, while for studies in mice, 4°C is typically used to model cold conditions, with 22°C as the normal control temperature[1]. To adapt to various environments, mammals have developed a wide array of complex and effective physiological and behavioral mechanisms, collectively known as phenotypic plasticity[2-3]. Environmental temperature, particularly cold exposure, significantly influences phenotypic plasticity[4-5]. The challenges posed by winter conditions require mammals to adopt various physiological and behavioral strategies for survival[6-8], with adipose tissue playing a central role.

Historically regarded as a passive energy storage organ, adipose tissue is now recognized as a dynamic regulator of systemic metabolism, largely due to the discovery of adipokines[9-10]. Adipose tissue consists of three main types: white, brown, and beige fat. White adipose tissue (WAT), characterized by large unilocular lipid droplets[11], stores excess energy as triglycerides and mobilizes it *via* lipolysis during energy deficiency[12]. Additionally, WAT plays a crucial role in maintaining glucose, lipid, and energy homeostasis, acting as a communicator between adipocytes and other tissues

involved in metabolic regulation[10]. Cold tolerance in mammals is closely linked to WAT function, though the underlying mechanisms remain unclear. Identifying key cold-responsive genes and pathways in white adipocytes could be valuable for diagnosing and treating cold-related diseases, such as cardiovascular disease and hypertension[13].

In this study, we analyze cold-induced differential gene expression in mice using the Gene Expression Omnibus (GEO) database, identify and validate hub genes, and conduct pathway enrichment analysis. This approach aims to shed light on the cold tolerance mechanisms in animals and to identify potential diagnostic and therapeutic markers for cold-related diseases.

2 Methods and materials

2.1 Data source

The microarray data set GSE148361 was obtained from the GEO database. This dataset is based on the GPL17400 [MoGene-2_1-st] Affymetrix Mouse Gene 2.1 ST Array (transcript [gene] version). For this study, we used samples from the GSE148361 dataset, which includes 4 epididymal WAT samples from C57BL/6 mice exposed to 4°C (cold exposure) and 4 samples from mice exposed to 22°C (control temperature). Additionally, we used wild-type and cold-exposed white adipose samples from the differentially expressed genes (DEG) 110420 dataset as an external validation set.

2.2 Identification of DEGs

DEGs between cold-exposed and control samples were identified using the "LIMMA" R package. A false discovery rate (FDR)-adjusted *P*-value (adj. *P*) of < 0.05 was considered significant. The results of the DEG analysis were visualized using heatmaps and volcano plots. LIMMA represents a library for the analysis of gene expression microarray data, especially the use of linear models for analyzing designed experiments and the assessment of differential expression.

2.3 Gene ontology and Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis

To identify the enriched biological processes, cellular components, and molecular functions (MF), the intersection of DEGs and cold-response genes (CRGs) was determined using the "VennDiagram" R package. The resulting set of DECRGs was subjected to Gene Ontology (GO) and KEGG pathway enrichment analysis using the "ClusterProfiler" R package[14]. GO terms were categorized into three groups: biological process (BP), cellular component (CC), and MF. KEGG enrichment analysis was performed to identify relevant metabolic and signaling pathways.

2.4 Construction of protein-protein interaction (PPI) network

PPI network for the 208 DEGs was constructed using the STRING database[15]. The network was further analyzed using the MCODE plugin in Cytoscape, with the following parameters: degree cutoff = 2, maximum depth = 100, k-core= 2, and node score cutoff = 0.2. The hub genes within the PPI network were identified, and the interactions between these genes were visualized using Cytoscape.

2.5 Gene set enrichment analysis (GSEA)

GSEA was performed to assess the functional pathways associated with the hub genes. Hallmark gene sets were obtained from the Molecular Signatures Database (MSigDB). Pathways with an adjusted *P*-value of < 0.05 were considered statistically significant.

2.6 Construction of regulatory network

To explore the regulatory relationships, potential miRNAs and upstream transcription factors (TFs) that could regulate the hub genes were predicted using the miRNet database[16]. The miRNA-TF-hub gene regulatory network was constructed and visualized using Cytoscape.

3 Results

3.1 Identification of DEGs

In this study, a total of 208 DEGs were identified based on an adjusted *P*-value < 0.05 and a fold change > |0.8|. Among these, 137 genes were upregulated, and 71 were downregulated in cold-exposed WAT compared to control samples. The DEGs are visualized in the volcano plot (Fig. 1A) and heatmap (Fig. 1B).

3.2 Functional enrichment analysis of DEGs

To explore the cold-related pathways, GO and KEGG enrichment analyses were performed on the 208 DEGs. For BP, DEGs were significantly enriched in nucleotide metabolic processes and purine-containing compound processes (Fig. 2A). In terms of CC, DEGs were associated with condensed chromosomes and mitochondrial protein-containing complexes (Fig. 2B). For MF, DEGs were involved in proton transmembrane transporter activity and oxidoreductase activity, acting on aldehydes (Fig. 2C). KEGG enrichment analysis revealed that these DEGs were significantly involved in biosynthesis of unsaturated fatty acids and pyruvate metabolism (Fig. 2D).

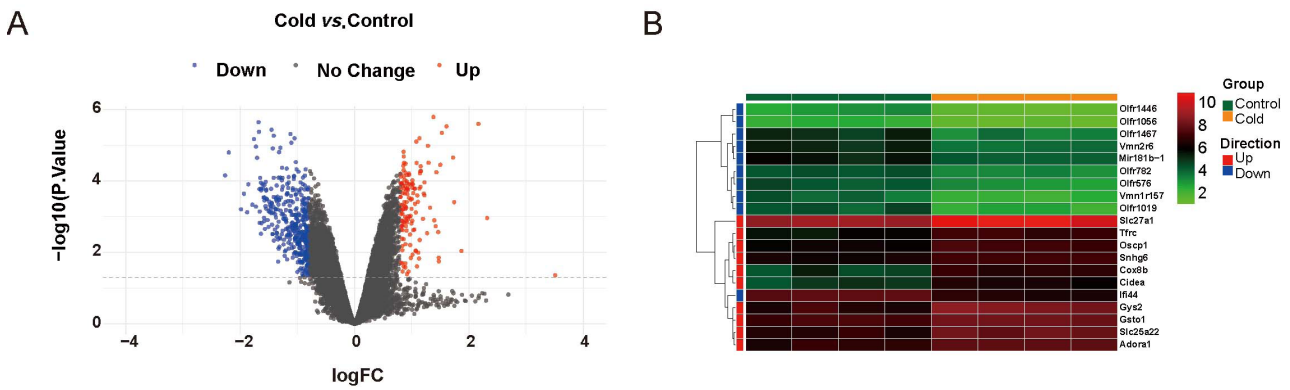

Fig. 1 Differential expressed genes identified using LIMMA R package in epididymal white adipose tissue samples between cold exposure and normal conditions with the data set GSE148361

(A) Volcano plot of the differential expressed genes, with blue and red colors representing downregulated and upregulated genes, respectively. (B) Heatmap for the top 10 upregulated genes and top 10 downregulated genes as sorted by adjusted p-value. LIMMA: a library for the analysis of gene expression microarray data, especially the use of linear models for analyzing designed experiments and the assessment of differential expression.

3.3 Identification of hub genes

The PPI network for the 208 DEGs was constructed using the STRING database (Fig. 3). Using the MCODE plugin in Cytoscape, 12 hub genes were identified: *Col1a2, Vcan, Ifi203, Iigp1, Lum, Gbp7, Ifi44, Cxcl9, Gbp3, Col3a1, Col1a1*, and *Rtp4* (Fig. 4A). The expression of these hub genes in the validation set is shown in Fig. 4B. These genes were found to be significantly upregulated in cold-exposed WAT compared to the control group ($P < 0.05$).

3.4 External validation of hub genes

To validate the accuracy of the identified hub genes, an external validation set (GSE110420) was used. The differential expression of the hub genes was assessed in WAT from 5 cold-exposed mice compared to normal controls. As shown in Fig. 5A, Col1a1, Col1a2, Col3a1, Gbp7, Ifi203, Ifi44, Vcan, and Rtp4 were all upregulated under cold exposure. Among them, the expression differences of Col1a1, Vcan, Ifi203, and Rtp4 were statistically significant ($P < 0.05$). These four genes (Col1a1, Vcan, Ifi203, and Rtp4) were selected as key genes for cold exposure.

3.5 GSEA

To further explore the function of the hub genes, GSEA was performed. The high-expression cohorts of Col1a1, Vcan, Ifi203, and Rtp4 were found to be significantly enriched in pathways related to ECM-receptor interactions, cytokine-cytokine receptor interactions, and Parkinson's disease (Fig. 6A-D). This suggests that cold exposure interacts with the extracellular matrix (ECM) and is related to Parkinson's disease, among other pathways.

3.6 Construction of TF-miRNA-diagnostic gene network

A regulatory network involving TFs and miRNAs was constructed. In total, 4 hub genes, 93 miRNAs (*e.g.*, mmu-miR-741-3p, mmu-miR-882, mmu-miR-185-5p, mmu-miR-3473a, mmu-miR-128-3p, mmu-miR-340-5p, mmu-miR-29a, mmu-miR-188-5p, mmu-miR-696, mmu-miR-122-5p, mmu-miR-23b-3p, mmu-miR-762, mmu-miR-133b-3p, mmu-miR-106a-5p, mmu-miR-1943-5p, *etc.*), and 1 transcription factor (Jun) were included in the TF-miRNA-diagnostic gene network (Fig. 7).

4 Discussion

Cold exposure has been identified as a key factor in the pathophysiology of several diseases. The aim of this study was to investigate genes associated with cold exposure and their potential implications. A total of 208 genes related to cold exposure were identified and subjected to GO and KEGG enrichment analyses, utilizing information from online databases. The enrichment analysis revealed that these genes were involved in nucleotide metabolic processes and purine-containing compound processes, which are crucial for regulating metabolic processes and maintaining temperature homeostasis. Additionally, these genes were implicated in regulating proton transmembrane transporter activity and oxidoreductase activity, which are involved in enzyme complex synthesis.

The PPI network analysis identified 12 hub genes (Col1a1, Col1a2, Col3a1, Gbp7, Ifi203, Ifi44, Vcan, Lum, Gbp3, Cxcl9, Rtp4, and Iigp1), all of which were significantly upregulated in response to cold exposure. These hub genes were further vali-

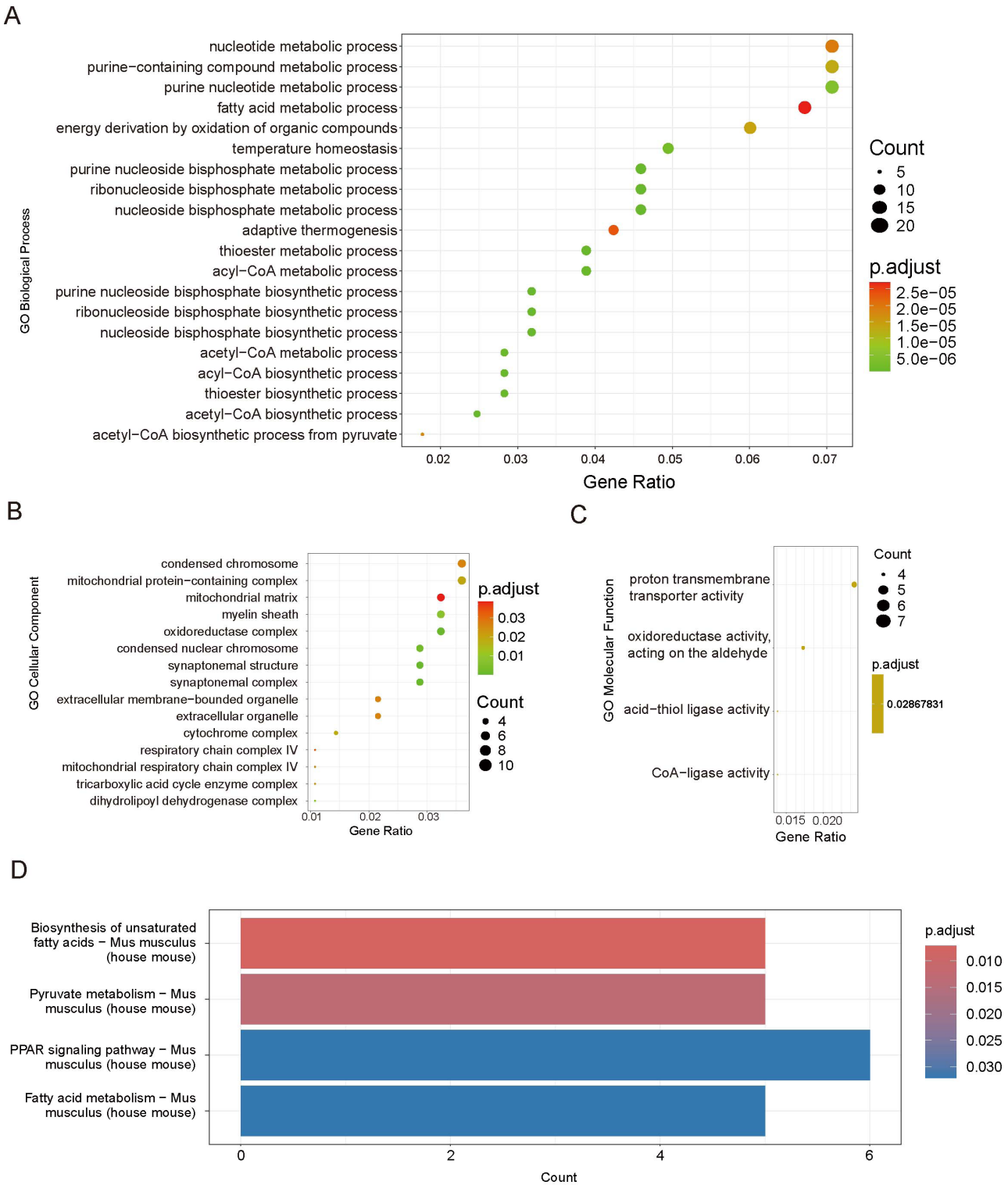

Fig. 2 Enrichment analysis of differentially expressed metabolism-related genes

(A) biological process (BP) GO enrichment of differentially expressed genes (DEGs). (B) BP GO enrichment of DEGs. (C) molecular functions (MF) GO enrichment of DEGs. (D) KEGG enrichment of DEGs.

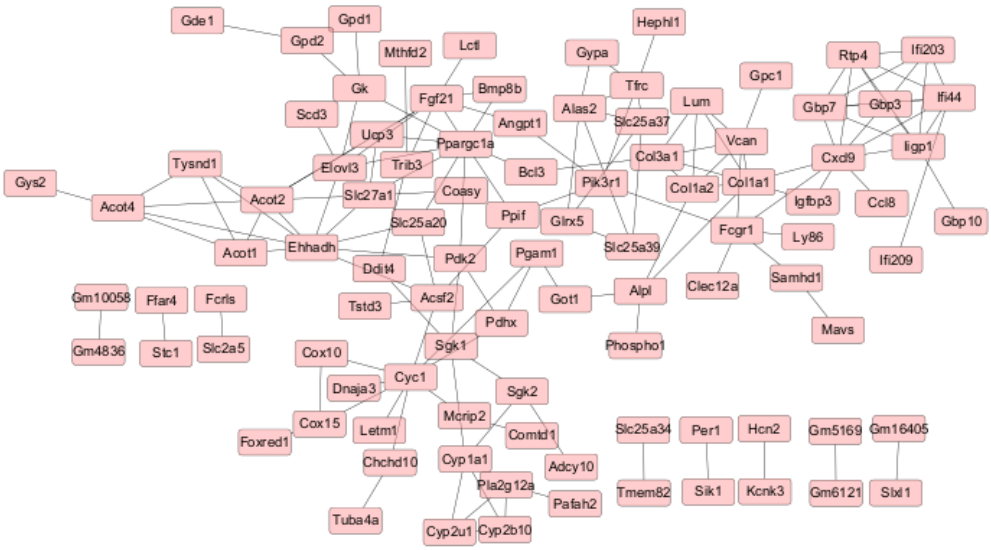

Fig. 3 Diagram illustrating the protein-protein interaction (PPI) network of DEGs

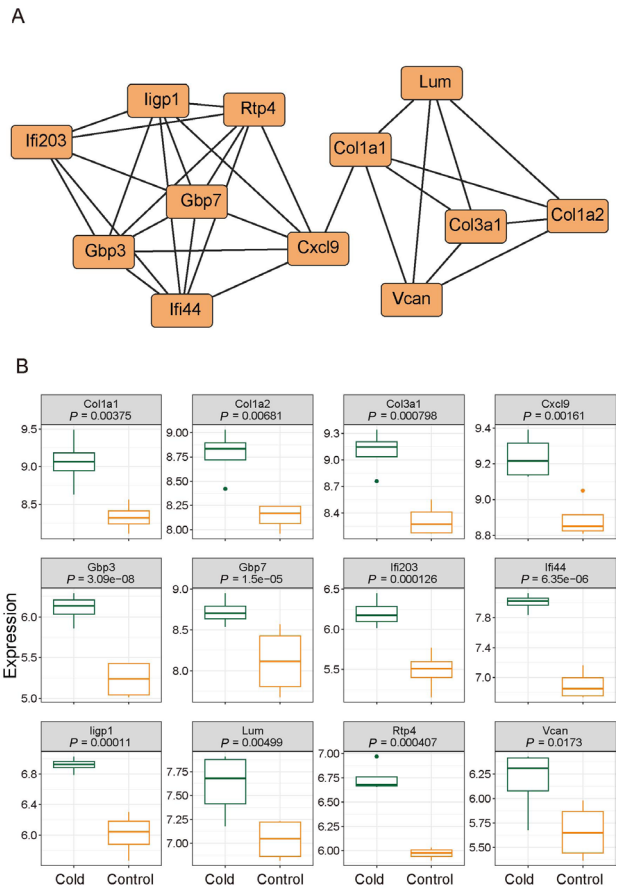

Fig. 4 MCODE identifies hub genes and their expression

(A) MCODE network diagram of hub genes from core module analyzed using MCODE. (B) Differential expression of hub genes in GSE45642.

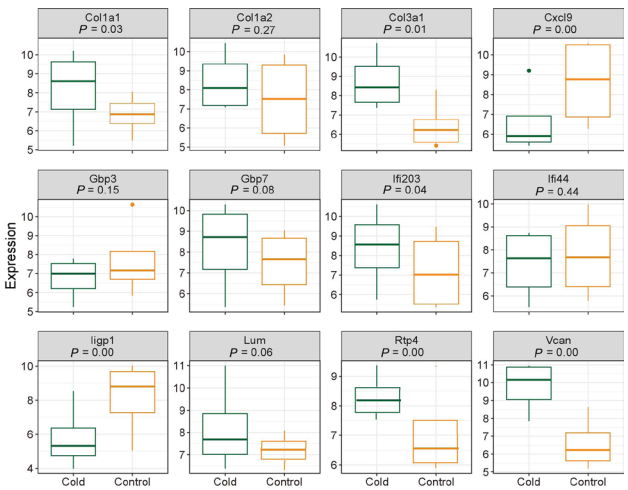

Fig. 5 The expression of hub genes

Differential expression of Hub genes between two groups in external validation set GSE110420.

dated in an external dataset, where four genes (Col1a1, Vcan, Ifi203, and Rtp4) showed consistent expression patterns and statistically significant differences, confirming their relevance to cold exposure.

Col1a1, a member of the collagen family, is involved in epithelial-mesenchymal transition and is highly expressed in various cancers. It regulates several cellular processes, including proliferation, metastasis, apoptosis, and cisplatin resistance[17]. Previous studies have shown that the stability of collagen is sensitive to temperature. For example, Col1a1 expression is normal at 37°C but abnormal at 22°C. While the relationship between Co-

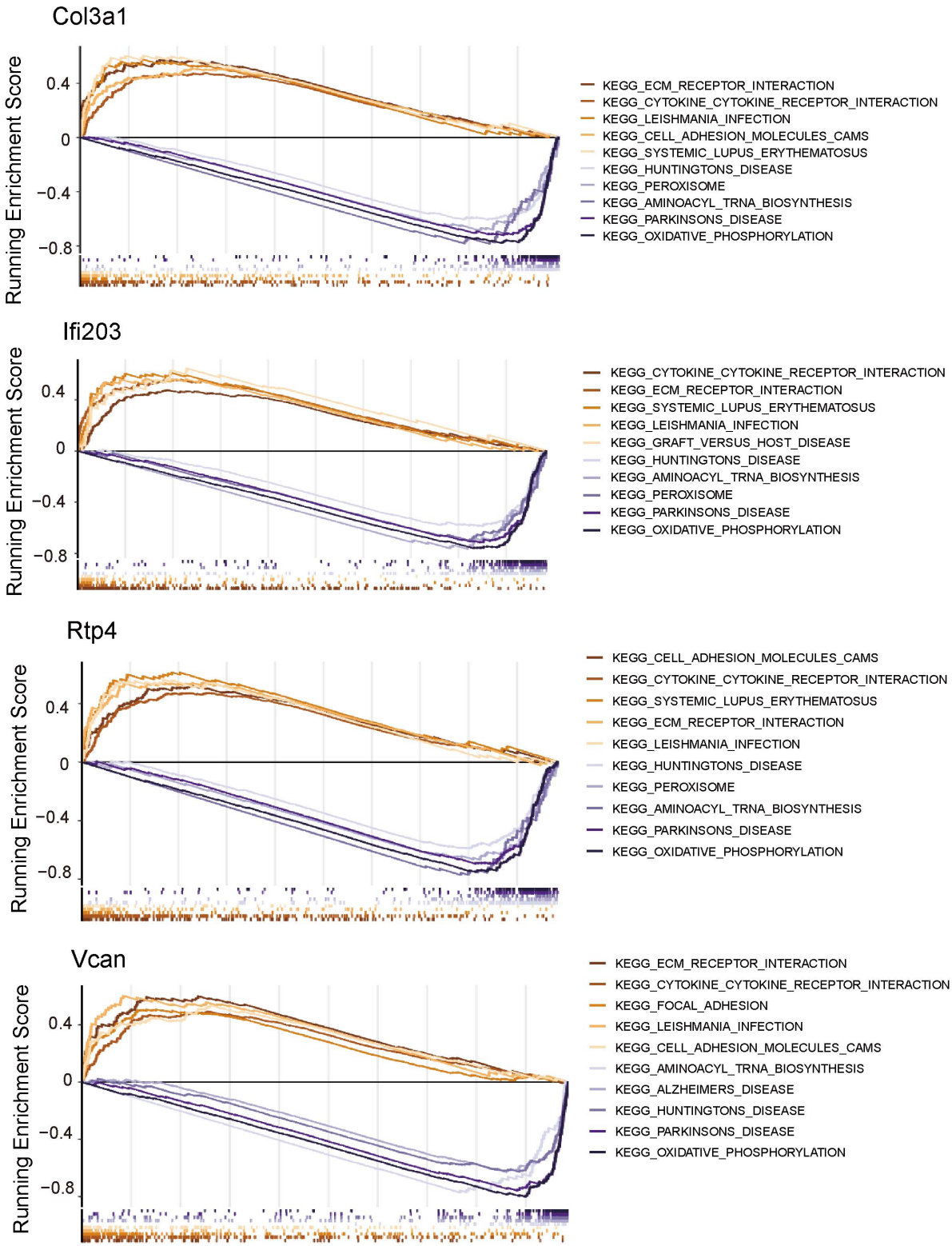

Fig. 6 Gene set enrichment analysis (GSEA) of hub genes

(A) GSEA pathways of Col3a1. (B) GSEA pathways of Ifi203. (C) GSEA pathways of Rtp4. (D) GSEA pathways of Vcan.

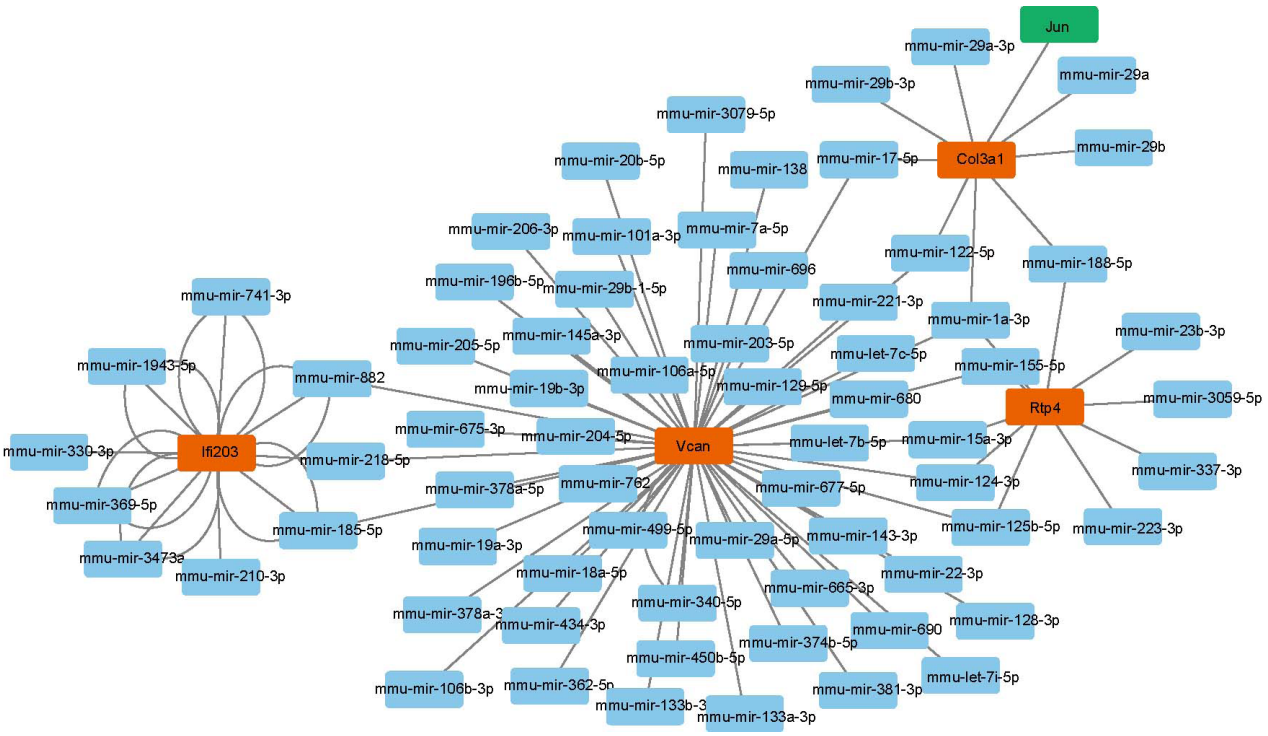

Fig. 7 Construction of the miRNA-gene-transcription factor regulatory network

Transcription factors (TFs) are represented by green color, miRNAs by bule, and hub genes by red.

l1a1 and cold exposure has been explored in other contexts, its expression in adipose tissue under cold conditions had not been studied prior to our work[18]. Our study is the first to demonstrate differential expression of Col1a1 in adipose tissue at 22°C and 4°C, highlighting its potential role in cold adaptation.

Ifi203, a member of the interferon-inducible protein family, has not previously been linked to temperature regulation[19]. Our findings suggest that Ifi203 expression is altered during cold exposure, providing novel insights into its potential involvement in temperature adaptation mechanisms.

VCAN encodes the protein versican, a proteoglycan that is a major component of the ECM. Versican plays a crucial role in cell adhesion, which may be modulated by temperature changes[20]. Our study suggests that VCAN may be involved in ECM-related pathways during cold exposure.

RTP4, a member of the receptor transporter protein family, is known to play a role in the surface expression of taste receptors and has been identified as a chaperone for opioid receptors[21-23]. However, its role in cold exposure has not been explored in previous studies. Our research reveals that RTP4 is significantly upregulated in response to cold, suggesting its involvement in temperature regulation processes.

The pathways identified in our study, such as ECM receptor interactions, cytokine-cytokine receptor interactions, and Parkinson's disease-related pathways, are all associated with cold exposure. A study by Liu *et al.*[24] identified ECM receptor interaction, glycerolipid metabolism, regulation of autophagy, and focal adhesion as critical pathways for cold tolerance in L. polyactis[24]. While our findings align with these results, the involvement of other pathways in cold exposure, such as those related to Parkinson's disease, is novel and warrants further investigation.

Our study focused on the bioinformatics analysis of cold-related genes, providing valuable insights into their potential roles in cold adaptation and associated biological processes. However, several limitations should be acknowledged. First, the data used in the analysis were derived from publicly available databases, which may contain inherent biases or incomplete annotations. Second, although in silico methods were extensively utilized to predict gene functions and regulatory networks, experimental validation is still required to confirm these findings. Future studies integrating multi-omics data and experimental approaches are needed to address these limitations and deepen our understanding of cold-related genes.

In conclusion, our study establishes a clear connection between cold exposure and gene expression changes in adipose tissue.

The identified genes may serve as potential biomarkers for cold exposure and provide a foundation for future studies on cold tolerance mechanisms. These findings can guide further experimental investigations into the pathophysiology of cold-related diseases and inform clinical approaches to managing cold-induced stress.

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Author contributions

Wang X conceived and designed the study. Hu H B prepared the figures and tables. Zhang Y wrote the manuscript. All authors contributed to the revision of the manuscript and approved the final submitted version.

Source of funding

Not applicable.

Ethical approval

The datasets used in this study are publicly available from the Gene Expression Omnibus (GEO) database, and the studies included in these datasets have received ethical approval. The data are freely accessible for research purposes, and the patients involved in the original studies have provided informed consent. As our study is based on open-source data, there are no ethical concerns or conflicts of interest associated with this research.

Informed consent

Not applicable.

Conflict of interest

The authors declare that there is no potential conflict of interest regarding this research.

Data availability statement

The datasets generated and/or analyzed during the current study are available in the GEO repository (GEO Accession viewer [nih. gov]).

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