Original Article

CCNB1 may as a biomarker for the adipogenic differentiation of adipose-derived stem cells in the postoperative fat transplantation of breast cancer

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Background: Adipose-derived stem cells (ADSCs) are closely associated with the survival rate of transplanted fat in breast reconstruction after breast cancer surgery. Nevertheless, the intrinsic mechanisms regulating ADSCs adipogenic differentiation remain ambiguous. The aim of our study was to explore the relevant genes and pathways to elucidate the potential mechanisms of adipogenic differentiation in ADSCs.

Methods: The Gene Expression Omnibus (GEO) dataset GSE61302 was downloaded and analyzed to identify differentially expressed genes (DEGs). Key genes and signaling pathways were obtained through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional and enrichment analysis. Protein-protein interaction (PPI) network and hub gene analyses were performed with the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database and Cytoscape software. Finally, the transcription levels of hub genes in the adipogenic differentiated group and undifferentiated group of ADSCs were compared via real-time quantitative polymerase chain reaction (RT-qPCR).

Results: In total, 1,091 DEGs were identified through bioinformatics analysis of the adipogenic differentiated group and undifferentiated group. If was then found that the 10 downregulated key genes, *CCNB1*, *NUSAP1*, *DLGAP5*, *TTK*, *CCNB2*, *KIF23*, *BUB1B*, *CDC20*, *CDCA8*, and *KIF11* may play important roles in the adipogenic differentiation of ADSCs. Subsequent *in vitro* experimental verification also revealed that the messenger RNA (mRNA) expression levels of cyclin B1 in adipogenic differentiated cells and undifferentiated cells were significantly different at the early stage ($P<0.05$), but there was no significant difference at the late stage ($P>0.05$).

Conclusions: As a key gene, *CCNB1* might be a potential biomarker in the adipogenic differentiation of ADSCs at the early stage.

Keywords: Adipose-derived stem cells (ADSCs); bioinformatics analysis; adipogenic differentiation; adipogenesis; breast cancer

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Introduction

Breast disease is one of the most common illnesses in women and includes both benign lesions and malignant tumors (1,2). Although surgery is the primary treatment mode for breast cancer, millions of women experience breast defects after surgery, and thus one urgent issue is adequate breast reconstruction to improve the quality of life of women diagnosed with breast cancer (3). With the development of medical technology, autologous fat grafting has gradually become the main surgical option for treating breast defects after breast cancer surgery (4). The rate of breast reconstruction among women varies across different countries but has continued to rise (5,6). Although autologous fat grafting is a common means of treating of breast defects after breast cancer surgery; however, this method involves a few drawbacks, such as the unknown chance of tumor recurrence, the damage to the donor site, and an unsatisfactory survival rate of fat grafting, which greatly reduces the patient's postoperative satisfaction and quality of life (7-10).

Breast tissue engineering after breast cancer surgery is applied to help alleviate the shortcomings of natural breast transplants by developing substitutes (11,12). In addition to the ability to form on demand, the tissue should be simple to use and prepare. For the past few years, considerable progress has been made in the study of human adipose-derived stem cells (ADSCs) (13), and many studies have shown that ADSCs have the ability to differentiate into adipocytes, contributing to fat tissue formation. And the expression of specific biomarkers like adiponectin and perilipin is used to confirm successful ADSCs adipogenic differentiation (14,15). Due to the properties of adipogenesis, ADSCs have significant value in clinical application in breast tissue engineering after breast cancer surgery, being practicable for the reconstruction of the breast with autologous cells. The transplantation of human ADSCs has thus attracted much attention as a promising therapeutic approach in recent years (16-20). Previous studies have shown that many transcription factors and cytokines regulate adipogenic differentiation of ADSCs, such as the CDK5/FOXC2, ERK1/2-PPARγ, and MAPK signaling pathways (21-26). Despite ADSCs becoming a research hotspot in breast tissue engineering after breast cancer surgery due to the abundance of their supply and high availability, there is still some uncertainty regarding the basic biological mechanism behind the adipogenic differentiation of ADSCs, whether there may be additional transcriptional regulators and epigenetic factors that influence ADSCs differentiation that are not yet fully understood (27-29). The impact of the microenvironment on ADSCs adipogenic differentiation, including the role of neighboring cells and extracellular matrix (ECM) components, requires further exploration. At last, ADSCs are a heterogeneous population, and understanding the factors that influence the variability in their adipogenic potential is an area that warrants more research (30).

With the rapid development of microarray chromatin immunoprecipitation (ChIP) and sequencing technology as well as the growing application of ADSCs in the field of clinical medicine, it is necessary to conduct bioinformatics studies related to ADSCs in order to further elucidate the specific mechanism related to the adipogenic differentiation of ADSCs. Bioinformatics analysis enables the integration of diverse datasets from various studies, allowing researchers to combine information on gene expression and signaling pathways. This integration can provide a more holistic view of the molecular mechanisms to understand ADSCs adipogenic differentiation. Furthermore, bioinformatics tools can be used to predict and analyze transcription factor binding sites, enhancers, and other regulatory elements in the genomic regions associated with adipogenic differentiation. Indeed, as the molecular biological mechanisms of adipogenic differentiation are still poorly understood, in this study, bioinformatics methods were used to screen the differentially expressed genes (DEGs) and

Highlight box

Key findings

- The results of this study indicate that *CCNB1* gene downregulation is pronounced in the early stage of adipogenic differentiation.

What is known and what is new?

- Adipose-derived stem cell differentiation includes distinct gene regulation.
- In our study, a combination of bioinformatics analysis and experimental validation was used to explore how gene behavior varies over time during adipogenic differentiation.

What is the implication, and what should change now?

- Our results suggest that the *CCNB1* gene can be used as potential biomarker for reflecting adipogenic differentiation and may have clinical value and feasibility in evaluating the postoperative fat transplantation of breast cancer. Moreover, *CCNB1* gene may be useful in future work involving the clinical treatment of patients of breast cancer. Moreover, bioinformatics analysis combined with experimental validation has greater accuracy in identifying important genes related to adipogenic differentiation.

their involved signaling pathways during the adipogenic differentiation of ADSCs in order to clarify the molecular biological mechanism of potential key genes during the adipogenic differentiation of ADSCs. Initially, we identified the DEGs from one microarray dataset selected from the Gene Expression Omnibus (GEO) database, and then a bioinformatics approach was used to analyze the signaling pathway and to construct a protein-protein interaction (PPI) network for targeting hub genes. Finally, *in vitro* experiments were conducted to verify the related genes. Ultimately, by mining the data related to the adipogenic differentiation of ADSCs in the GEO database, we identified the potential key genes and the pathways involved in the adipogenic differentiation of ADSCs. Our findings contribute to further exploring the molecular biological mechanism related to the occurrence and development of adipogenic differentiation of ADSCs and to providing a new direction for the future clinical application of breast reconstruction after breast cancer surgery. We present this article in accordance with the MDAR reporting checklist (available at https://gs.amegroups.com/article/view/10.21037/gs-23-493/rc).

Methods

Study design

GEO dataset GSE61302 was downloaded and analyzed to identify DEGs, then relevant signaling pathways were found by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. Next, to establish PPIs network, so hub genes were obtained by Cytoscape software. *In vitro*, preadipocytes were cultured and identified, RNA isolation from differentiated and undifferentiated group. Finally, verification of the messenger RNA (mRNA) expression of hub genes at days 7 and 21.

Data collection

GEO (https://www.ncbi.nlm.nih.gov/geo) is an international, public database for functional genomics that includes high-throughput resources, gene expression data, ChIP sequencing (ChIP-seq), and microarrays. We obtained the transcript profile data on adipogenic differentiation from GSE61302 (31). The GSE61302 dataset contains five undifferentiated samples (GSM1501805, GSM1501806, GSM1501807, GSM1501808, and GSM1501809) and 10 adipogenic differentiated samples (GSM1501795, GSM1501796, GSM1501798, GSM1501799, GSM1501800, GSM1501801, GSM1501802, GSM1501803, GSM1501804, and GSM1501805). Based on the platform annotation information, probes were transformed into corresponding gene symbols.

Identification of DEGs

R software (The R Foundation for Statistical Computing, Vienna, Austria) was applied for data mining and statistical analyses. The "limma" package in R was subsequently used for removing batch effects and identifying DEGs, with those with a $\log_2$fold change ($\log_2$FC) >1 or <–1 and a P value <0.05 being considered significant.

GO and KEGG enrichment analysis

KEGG is a database resource for providing biological pathway information. GO is also a major bioinformatics database that for high-quality functional gene annotation and analyzing gene biological processes (BPs), molecular functions (MFs), and cellular components (CCs). GO and KEGG enrichment analyses of the identified DEGs were performed using the "clusterProfiler" 4.0 package in R (32-34). R software packages can be used to perform both enrichment analyses of genes and gene clusters and the visualization of gene profiles.

PPI network construction and module analysis

The PPI network was analyzed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; https://string-db.org). An interaction with a combined score >0.4 was chosen and used to construct a PPI network in Cytoscape software (version 3.9.1) (35). Cytoscape is an open-source platform for visualizing networks of molecular interaction (36). Dense connected regions were analyzed using the Cytoscape plug-in molecular complex detection (MCODE). Our selection criteria were as follows: degree cutoff =2, node score cutoff =0.2, max depth =100, and k-score =2 (37).

Selection and analysis of hub genes

The top 10 genes were obtained via the Matthews correlation coefficient (MCC) algorithm with the Cytoscape's plug-in cytoHubba.

Cell culture and adipogenic differentiation

According to a previously described method, preadipocytes were isolated and cultured from aseptic human adipose tissue. Tissue samples were obtained from the patients with benign breast disease from the Department of Breast Surgery, the First Affiliated Hospital of Nanjing Medical University, between August 2023 and October 2023. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (protocol code: 2022-NT-79). Informed consent was obtained from all participants involved in the study. Aseptic human adipose tissue was obtained and digested with 1 mg/mL of type I collagenase for 60 minutes, terminated with preadipocyte medium (PAM; ScienCell, Carlsbad, CA, USA), and then centrifuged at 1,500 rpm for 10 minutes. The cells were seeded in a 10-cm dish and incubated in a 37 ℃ incubator with 5% CO_2 for 24 hours, after which the medium was replaced. Cells cultured for 2–4 passages were used for seeding a 6-cm dish at a density of 20,000 cells/cm^2. The adipogenic differentiation medium (Haixing Biosciences, Suzhou, China) was substituted for the original medium when cell confluence was 80–90%. The medium was changed every 3 days. The adipogenic effects were identified via oil red O (ORO) staining at days 7 and 14.

ORO staining

Lipid droplets were detected with ORO (Sigma-Aldrich, St. Louis, MO, USA) staining. ADSCs were washed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for 60 minutes at room temperature; this was followed by another wash with PBS, after which the ADSCs were incubated with ORO staining solution for another 30 minutes at room temperature. Images were obtained using Olympus microscope.

RNA isolation and real-time quantitative polymerase chain reaction (RT-qPCR) analysis

Following the manufacturer's protocol, total RNA was isolated from undifferentiated and differentiated cells with TRIzol LS (Life Technologies, Carlsbad, CA, USA; Thermo Fisher Scientific, Waltham, MA, USA). Complement DNA (cDNA) was prepared with a HiScript III First Strand cDNA Synthesis Kit [+genomic DNA (gDNA) wiper] (Vazyme Biotech, Nanjing, China). Next, RT-qPCR was performed using ChamQ SYBR qPCR Master Mix (Vazyme Biotech). The reactions were conducted in the Roche Light Cycler 480 Real-Time PCR System (Roche Diagnostics), with the $2^{-\Delta\Delta Ct}$ method being used to calculate the expression level of genes in each sample. The primer sequences for RT-qPCR are presented in the supplementary information (*Table 1*).

Statistical analysis

The bioinformatics analyses were conducted using R v.4.1.3, with R packages include "limma", "dplyr", "ggplot2", and "clusterProfiler", among others. Statistical analysis with the paired Student *t*-test was performed with GraphPad Prism 9.0 software (GraphPad Software, La Jolla, CA, USA). All data are presented as the mean ± stand error of the mean (SEM). Values of $P<0.05$ were considered statistically significant. All experiments were repeated three times to ensure the accuracy of the results.

Results

Identification of DEGs

After normalization of the data, the analysis of DEGs was performed in the dataset that included 10 adipogenic differentiated samples and five undifferentiated control samples. A total of 1,091 DEGs were screened according to the criteria of false discovery rate (FDR) <0.05 and $\log_2$FC >1 or < –1. Among these genes, 382 genes were upregulated and 709 downregulated, as shown in the volcano plot in *Figure 1*.

GO and KEGG enrichment analyses of DEGs

In order to analyze the DEGs at the functional level, we performed functional enrichment analysis of the corresponding genes to determine the potential functions. The GO analysis results were mainly associated with leukocyte migration, cell chemotaxis, collagen-containing ECM, and glycosaminoglycan binding (*Figure 2A*). KEGG pathway analysis indicated that DEG enrichment was mainly related to viral protein interaction with cytokine and cytokine receptor, tumor necrosis factor (TNF) signaling pathway, and cytokine-cytokine receptor interaction (*Figure 2B*). In addition, the related pathways of upregulated genes in KEGG mainly included viral protein interaction

Table 1 Primer sequences for RT-qPCR

Gene symbol	Forward primers	Reverse primers
GAPDH	GGACCTGACCTGCCGTCTAG	GTAGCCCAGGATGCCCTTGA
NUSAP1	AGCCCATCAATAAGGGAGGG	ACCTGACACCCGTTTTAGCTG
CCNB1	AATAAGGCGAAGATCAACATGGC	TTTGTTACCAATGTCCCCAAGAG
DLGAP5	AAGTGGGTCGTTATAGACCTGA	TGCTCGAACATCACTCTCGTTAT
TTK	TCATGCCCATTTGGAAGAGTC	CCACTTGGTTTAGATCCAGGC
CCNB2	CCGACGGTGTCCAGTGATTT	TGTTGTTTTGGTGGGTTGAACT
BUB1B	AAATGACCCTCTGGATGTTTGG	GCATAAACGCCCTAATTTAAGCC
KIF23	AGTCAGCGAGAGCTAAGACAC	GGTTGAGTCTGTAGCCCTCAG
CDCA8	GCAGGAGAGCGGATTTACAAC	CTGGGCAATACTGTGCCTCTG
CDC20	GCACAGTTCGCGTTCGAGA	CTGGATTTGCCAGGAGTTCGG
KIF11	TCCCTTGGCTGGTATAATTCCA	GTTACGGGGATCATCAAACATCT

RT-qPCR, real-time quantitative polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

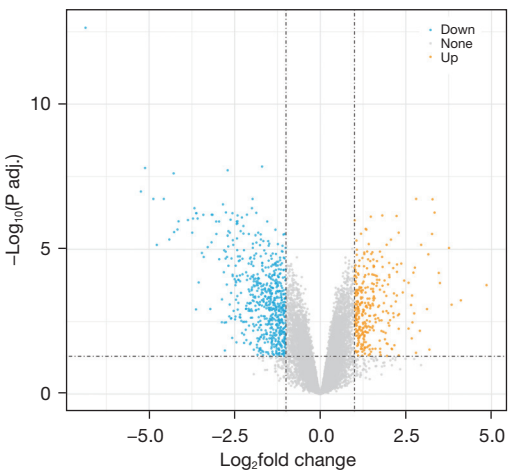

Figure 1 Identification of DEGs. The volcano plot shows the 1,091 DEGs. The gray part of the figure represents genes that were not differentially expressed, the yellow color indicates the upregulated genes, and blue indicates the downregulated genes. Adj., adjusted; DEG, differentially expressed gene.

with cytokine and cytokine receptor, TNF signaling pathway, cytokine-cytokine receptor interaction, and peroxisome proliferator-activated receptor (PPAR) signaling pathway (*Figure 2C*); meanwhile, the pathways associated with downregulated genes mainly included complement and coagulation cascades and C-type lectin receptor signaling pathway (*Figure 2D*).

PPI network construction and module analysis

Based on the STRING database, the PPI network of the DEGs was constructed. We used 382 upregulated DEGs and 709 downregulated DEGs to create the PPI network (*Figure 3A*). We also selected and analyzed the central dense connected regions according to the corresponding criteria, as these central node genes may potentially be relevant to regulating adipogenic differentiation (*Figure 3B*).

Selection and analysis of hub genes

The Cytoscape plug-in cytoHubba identified 10 hub genes. A centrality analysis of the nodes in the PPI network revealed that *NUSAP1*, *CCNB1*, *DLGAP5*, *TTK*, *CCNB2*, *KIF23*, *BUB1B*, *CDC20*, *CDCA8*, and *KIF11* were crucial genes (*Figure 4*). The gene symbols, abbreviations, and functions are shown in the supplementary information (*Table 2*).

Preadipocyte culture and observation

In our study, preadipocytes were successfully isolated using the conventional method, and the growth of cells was observed. Morphologically, the preadipocytes appeared spindle-shaped under microscopy and the differentiated group showed obvious lipid droplets with ORO staining at days 7 and 14 (*Figure 5*).

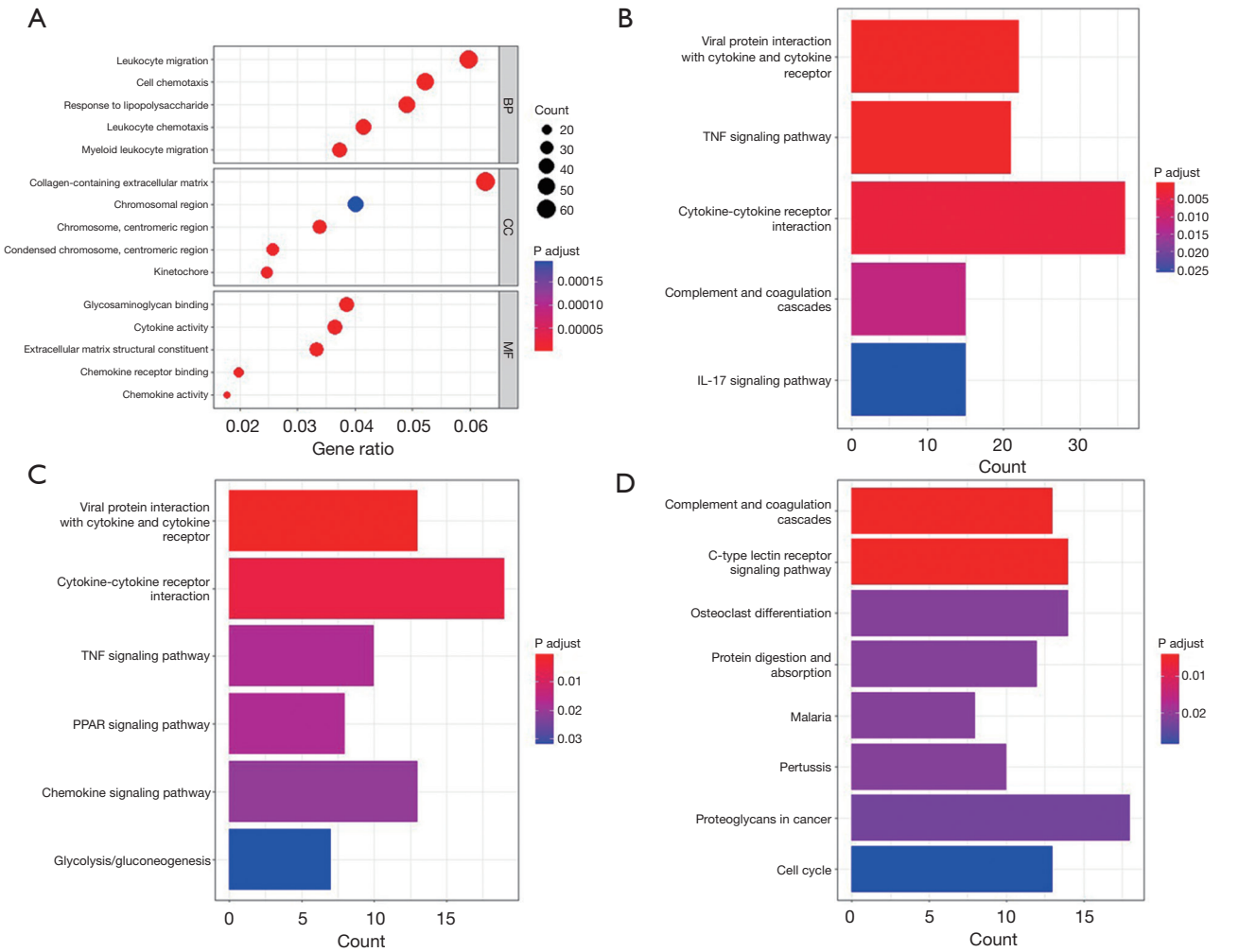

Figure 2 Enrichment analysis of the DEGs. (A) GO functional analysis results illustrating the important biological actions of the DEGs. (B) Results of the KEGG enrichment analysis of the DEGs. (C,D) Results of the KEGG enrichment analysis of the upregulated and downregulated DEGs. BP, biological process; CC, cellular component; MF, molecular function; TNF, tumor necrosis factor; IL, interleukin; PPAR, peroxisome proliferator-activated receptor; DEG, differentially expressed gene; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Verification of hub gene expression

To further validate the roles of hub genes in the differentiated and undifferentiated group as determined by the above-described data, the mRNA expression levels were examined with RT-qPCR at day 7 (early stage) and day 21 (late stage). The results indicated there to be no significant differences in *NUSAP1*, *CCNB1*, *DLGAP5*, *TTK*, *CCNB2*, *KIF23*, *BUB1B*, *CDC20*, *CDCA8*, or *KIF11* between the differentiated group and the undifferentiated group (P>0.05) at day 7; however, the mRNA expression levels of the adipogenic marker gene (*CCNB1*) in adipogenic cells were significantly different from those in the cells of the control group (P=0.03, P<0.05) (*Figure 6*). As time elapsed, we further found that after 21 days of adipogenic differentiation, there was no significant difference in the detection of 10 hub genes between the differentiated and undifferentiated groups (P>0.05) (*Figure 7*).

Discussion

In the field of breast tissue reconstruction for those who undergo breast cancer surgery, various biomaterials and

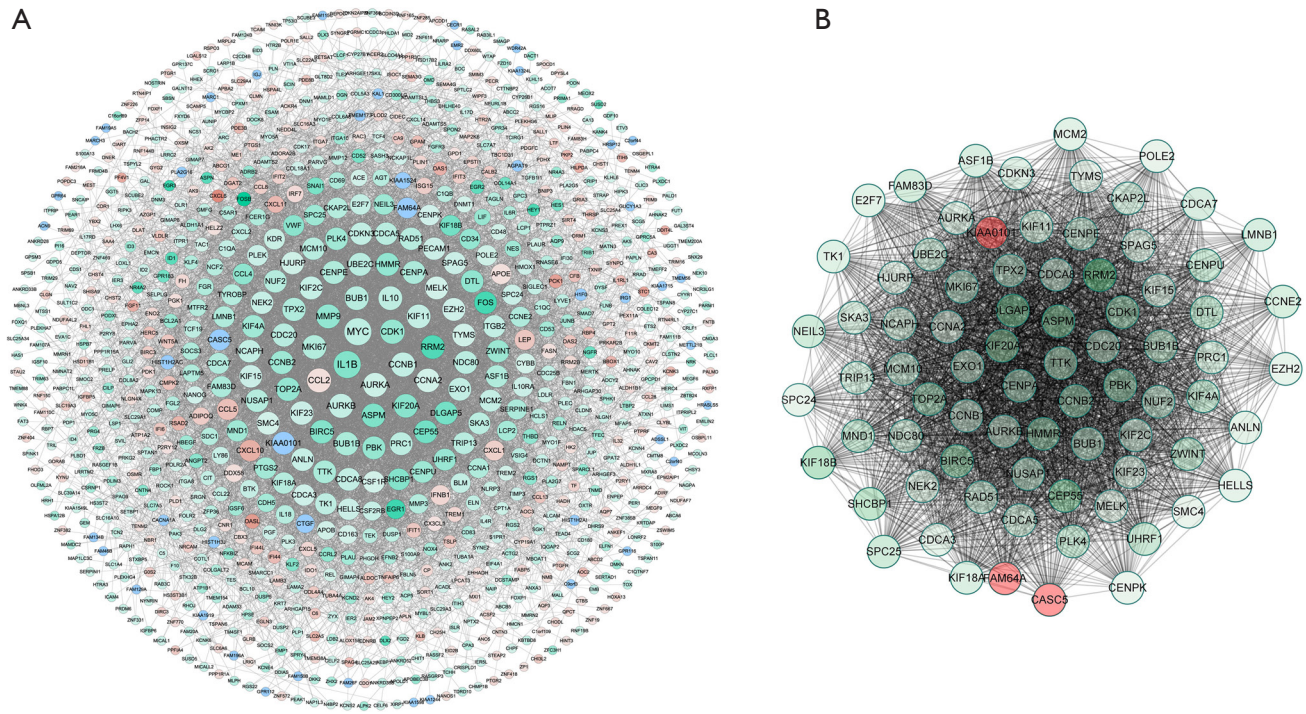

Figure 3 PPI network of the significant DEGs was constructed, and module analysis was completed. (A) The upregulated genes are marked in red, while the downregulated genes are marked in green. (B) The central densest connected regions (78 nodes, 2,746 edges) in the PPI network were identified with the Cytoscape plug-in MCODE. PPI, protein-protein interaction; DEG, differentially expressed gene; MCODE, molecular complex detection.

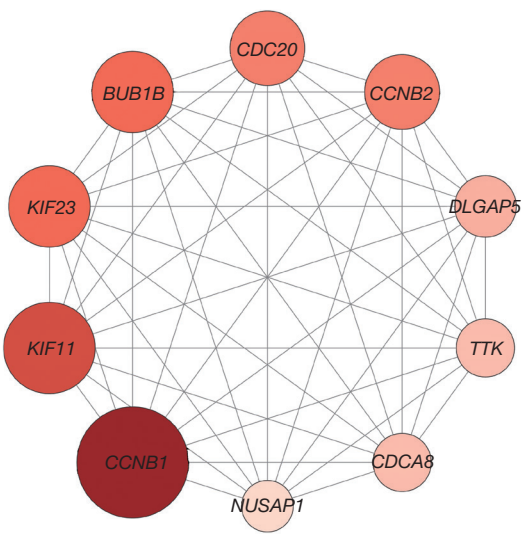

Figure 4 Ten hub genes were identified in the densest connected regions with the MCC algorithm using the cytoHubba plug-in of Cytoscape. The colors and font size of hub genes vary according to the degree value. MCC, Matthews correlation coefficient.

synthetic materials have been employed extensively in recent years (38-40). The self-renewal ability, proliferation potential, and multidirectional differentiation characteristics of ADSCs are of great value in regenerative medicine applications after breast cancer surgery (41-43). The easily accessible and large number of ADSCs make them a promising seed cell for breast tissue engineering after breast cancer surgery. But some factors can influence the effectiveness of ADSCs, such as donor variability, isolation and culture conditions, cell proliferation and viability, immunomodulatory properties and so on, leading to the delayed wound healing in regenerative medicine and low retention rate of adipose tissue in fat transplantation (44). Several studies have explored the mechanisms underlying the survival of adipocytes. For example, LRG-1-mediated suppression of the ADSCs apoptosis induced by hypoxia was mediated by the upregulation of *RAB31* expression in fat transplantation. Besides, browning of adipocytes in patients resulted in poorly survived fat grafts, because it had a

Table 2 Ten hub genes and their functions

Gene symbol	Description	Function
NUSAP1	Nucleolar and spindle associated protein 1	Microtubule-associated protein with the capacity to bundle and stabilize microtubules (by similarity)
CCNB1	Cyclin B1	Essential for the control of the cell cycle at the G2/M (mitosis) transition
DLGAP5	Discs large homolog associated protein 5	Potential cell cycle regulator that may play a role in carcinogenesis of cancer cells
TTK	TTK protein kinase	Probably associated with cell proliferation
CCNB2	Cyclin B2	Essential for the control of the cell cycle at the G2/M (mitosis) transition
BUB1B	BUB1 mitotic checkpoint serine/threonine kinase B	Essential component of the mitotic checkpoint
KIF23	Kinesin family member 23	Component of the centralspindlin complex that serves as a microtubule-dependent and Rho-mediated signaling required for the myosin contractile ring formation during the cell cycle cytokinesis
CDCA8	Cell division cycle associated 8	Major effector of the TTK kinase in the control of attachment-error-correction and chromosome alignment
CDC20	Cell division cycle 20	Required for full ubiquitin ligase activity of the APC/C and may confer substrate specificity upon the complex
KIF11	Kinesin family member 11	Motor protein required for establishing a bipolar spindle during mitosis

APC/C, anaphase promoting complex/cyclosome.

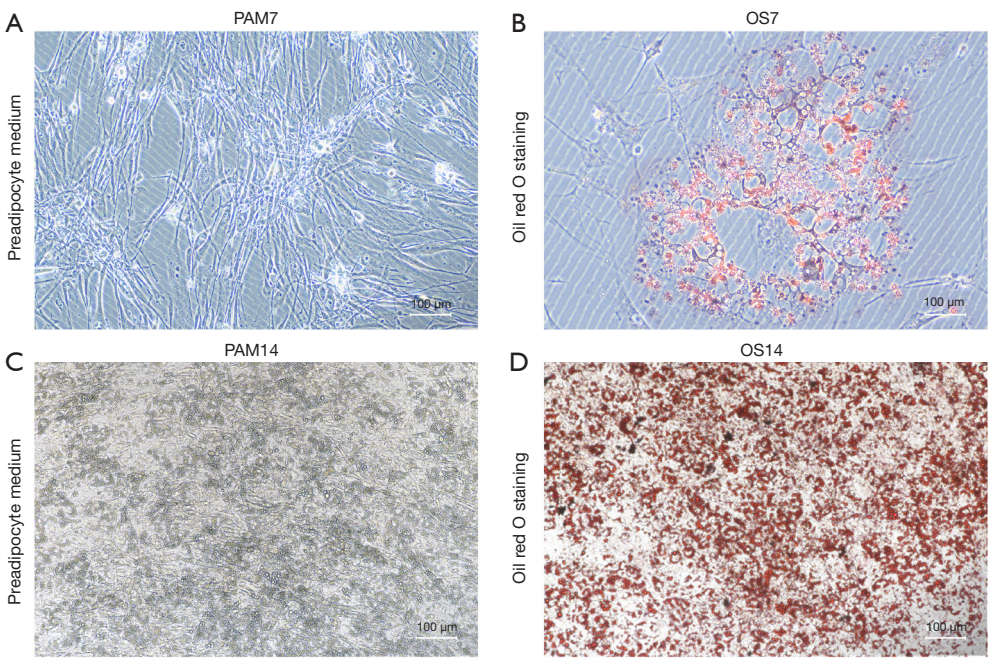

Figure 5 Observation of preadipocytes. (A) Observation of undifferentiated preadipocytes at day 7 under a 100× optical microscope. Scale bar: 100 μm. (B) Observation of differentiated preadipocytes incubated with ORO staining solution at day 7 under a 100× optical microscope. Scale bar: 100 μm. (C) Observation of undifferentiated preadipocytes at day 14 under a 100× optical microscope. Scale bar: 100 μm. (D) Observation of differentiated preadipocytes incubated with ORO staining solution at day 14 under a 100× optical microscope. Scale bar: 100 μm. PAM7 and PAM14: cells cultured in PAM for 7 and 14 days, respectively; OS7 and OS14: cells cultured in adipogenic differentiation medium for 7 and 14 days, respectively, and then incubated with ORO staining solution. PAM, preadipocyte medium; ORO, oil red O.

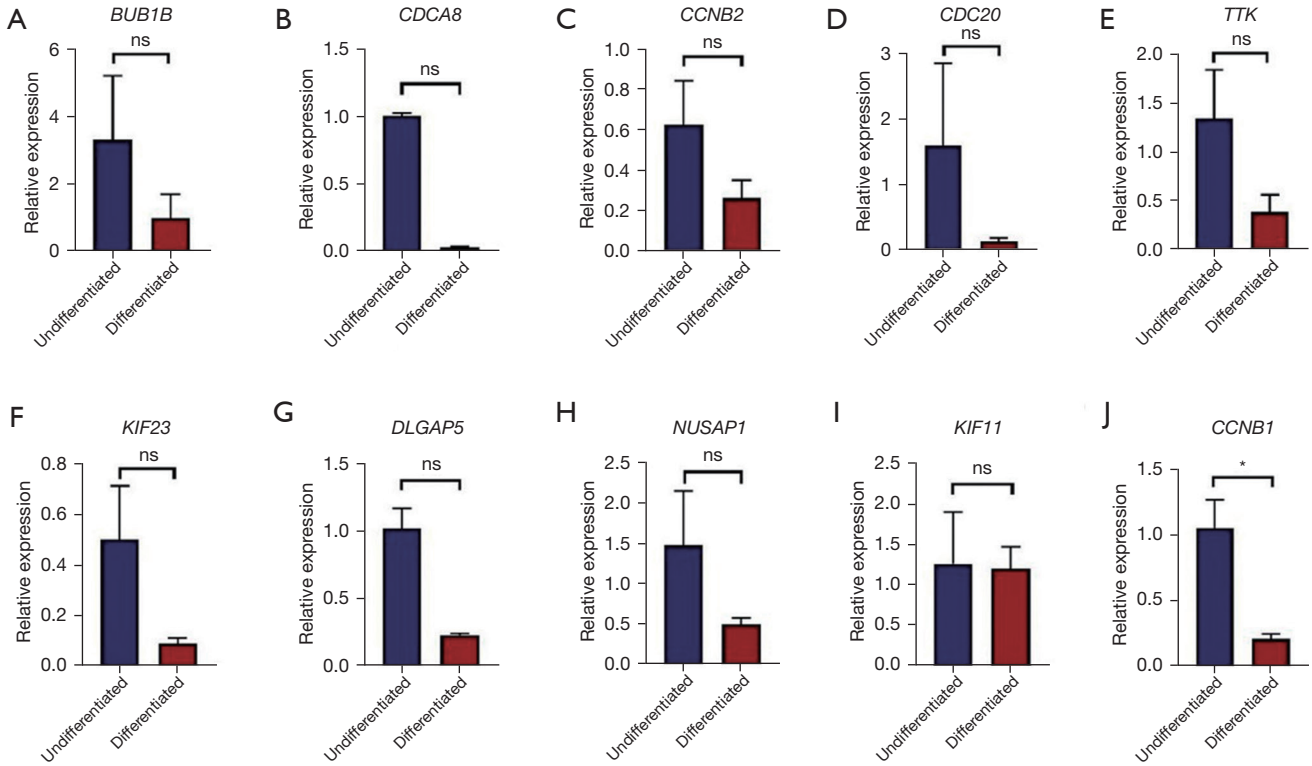

Figure 6 Verification of the mRNA expression of hub genes at day 7. (A-J) RT-qPCR analysis of mRNA expression of candidate genes (*NUSAP1*, *DLGAP5*, *TTK*, *CCNB2*, *KIF23*, *BUB1B*, *CDC20*, *CDCA8*, *KIF11*, and *CCNB1*). Statistical differences were measured with the Student *t*-test. *, P<0.05; ns, non-significant. (J) Verification of *CCNB1* showed differences between the differentiated and undifferentiated group in the early stage. mRNA, messenger RNA; RT-qPCR, real-time quantitative polymerase chain reaction.

higher gene expression level of uncoupling protein-1. Apart from the above-mentioned, A series of signaling pathways, epigenetic modifiers, and transcription factors play important roles in preadipocyte commitment and terminal differentiation into mature adipocytes. During the process, ADSCs can stimulate angiogenesis through differentiation into epithelial cells and release angiogenic factors by paracrine activity. Finally, dedifferentiation, which a mature adipocyte is reverted to an undifferentiated progenitor-like status under both physiological and pathological conditions, also has been reported as a mechanism explaining adipocyte plasticity (45-48). Therefore, the molecular mechanisms underlying the adipogenic differentiation of ADSCs still should be explored to facilitate the success of ADSCs-based therapies after breast cancer surgery.

In this study, we gathered and examined samples of ADSCs undergoing adipogenic differentiation from the GSE61302 dataset. Of the 1,091 DEGs that were evaluated in the dataset, 382 genes were upregulated and 709 genes were downregulated. Moreover, for a better understanding of the interactions between the identified DEGs, GO and KEGG enrichment analyses were performed. The GO analysis revealed that the 1,091 DEGs were mainly linked with leukocyte migration, cell chemotaxis, collagen-containing ECM, and glycosaminoglycan binding. A previous study has indicated that the ECMs from multiple sources include similar types of collagens but differ in the relative abundance of each; regardless, all of them are capable of inducing stronger osteogenesis and adipogenesis in mesenchymal stem cells (49). According to the KEGG pathway analysis in our study, the identified DEGs were related to viral protein interaction with cytokine and cytokine receptor, TNF signaling pathway, and cytokine-cytokine receptor interaction. Meanwhile, the pathways associated with upregulated and downregulated genes were TNF signaling pathway, cytokine-cytokine receptor

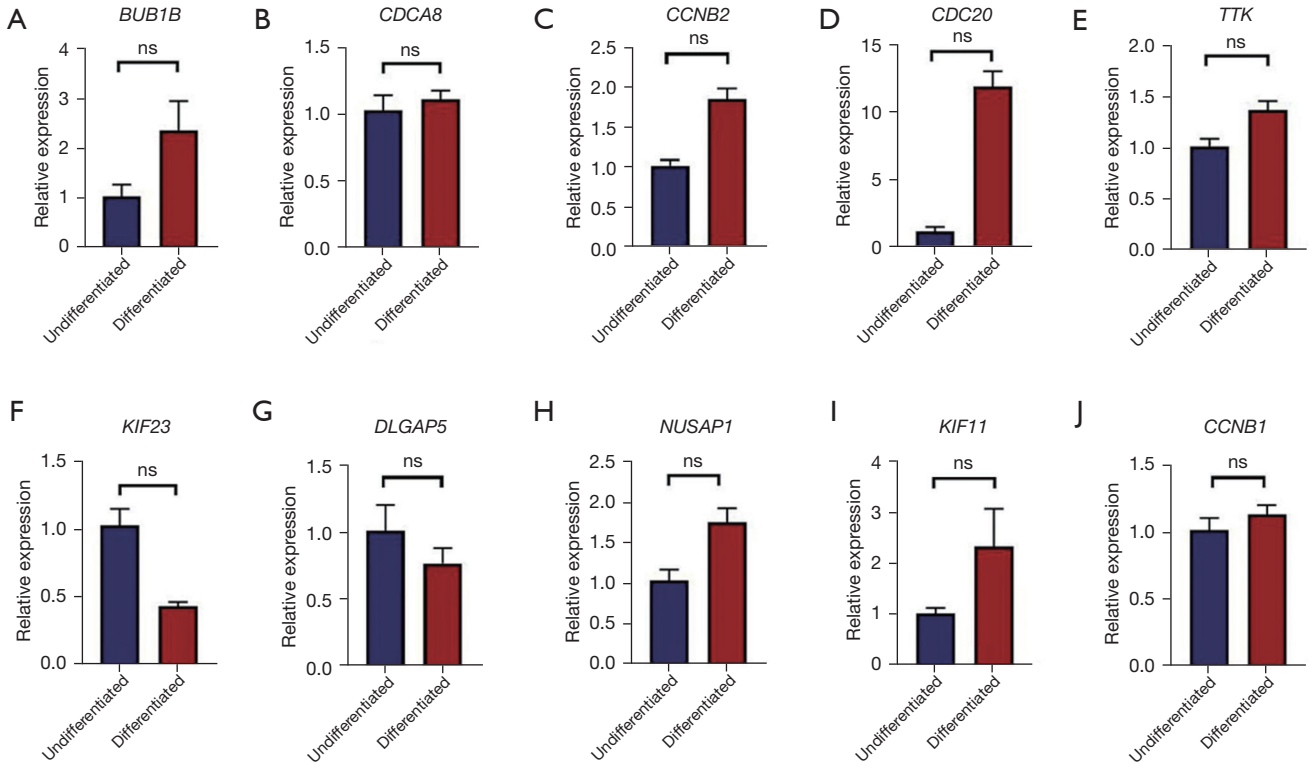

Figure 7 Verification of the mRNA expression of hub genes at day 21. (A-J) RT-qPCR analysis of mRNA expression of the candidate genes (*NUSAP1*, *DLGAP5*, *TTK*, *CCNB2*, *KIF23*, *BUB1B*, *CDC20*, *CDCA8*, *KIF11*, and *CCNB1*). Statistical differences were measured with the Student *t*-test. ns, non-significant. Verification of all hub genes indicated no significant differences between the differentiated and undifferentiated group in the late stage. mRNA, messenger RNA; RT-qPCR, real-time quantitative polymerase chain reaction.

interaction, PPARγ signaling pathway, complement and coagulation cascades, and C-type lectin receptor signaling pathway. Among these pathways, the TNF signaling pathway and the PPARγ signaling pathway are of particular importance, functioning as master transcriptional regulators for adipocyte differentiation. Indeed, PPARγ belongs to the nuclear hormone receptor superfamily of ligand-activated transcription factors, which is known to inhibit osteoblast differentiation and promote adipogenic differentiation (50,51).

Based on the centrality of the nodes in the PPI network, the *NUSAP1*, *DLGAP5*, *TTK*, *CCNB2*, *KIF23*, *BUB1B*, *CDC20*, *CDCA8*, *KIF11*, and *CCNB1* genes were among the significant DEGs found. Of these 10 hub genes, 1 gene, *CCNB1*, was downregulated in adipogenic differentiation. *CCNB1* is a protein-coding gene, and the protein encoded by this gene is a regulatory protein involved in mitosis and is necessary for proper control of the G2/M transition phase of the cell cycle. Among its related pathways are regulation of activated PAK-2p34 via proteasome-mediated degradation and loss of Nlp from mitotic centrosomes (52). Previous studies revealed that *CCNB1* can regulate the proliferation of bone marrow stem cells, and other animal experiments have also demonstrated that the increase and decrease of *CCNB1* gene expression level are related to the differentiation and proliferation of adipocytes (53,54).

However, previous study just showed that adipose ECM and microenvironment possess the capability to promote early adipogenesis, while the ECM alone cannot induce adipogenesis *in vivo*. ADSCs can adjust early adipogenesis with angiogenesis and tissue remodeling by directly differentiating into endothelial cells and regulating macrophage polarization, contributing to better long-term retention and greater tissue integrity (55). Few studies have explored the relationship between *CCNB1* and adipogenic differentiation in human ADSCs, and this association is still poorly understood. Therefore, this study combined public databases and biological experimental

techniques to investigate the genes related to adipogenic differentiation of human adipocytes. We discovered that the expression level of the *CCNB1* gene in the differentiated group was significantly different from that in the undifferentiated group ($P<0.05$) in the early stage, but no significant difference was observed in the late stage ($P>0.05$). Therefore, it is worth continuing to investigate the underlying mechanisms of these changes.

This study offers a range of different perspectives on the adipogenic differentiation of human adipocytes. First, microarray and bioinformatics analyses were applied to assess whole transcriptome changes between differentiated and undifferentiated cells, and a preliminary investigation of the related mechanism was also carried out. Second, the combination of bioinformatics methods and molecular biology experiments in this study not only compensates for the limitations of bioinformatics methods but also improves the accuracy of the molecular biology experiments.

However, there are still some limitations to this study. First, more datasets or samples could have been included in the examined cohort. Second, the results and conclusions of this study should be verified by more experiments in the future.

In summary, these results provide the basis for further study of the adipogenic differentiation of ADSCs. One focus of future studies could involve the search for other genes closely related to the adipogenic differentiation of stem cells in order to better understand the related mechanisms and their value for breast reconstruction after breast cancer surgery.

Conclusions

Based on a bioinformatics method, this study conducted a comprehensive analysis of DEGs between differentiated and undifferentiated ADSCs. The key genes, *CCNB1*, *NUSAP1*, *DLGAP5*, *TTK*, *CCNB2*, *KIF23*, *BUB1B*, *CDC20*, *CDCA8*, and *KIF11*, may be significantly related to the adipogenic differentiation of ADSCs. After further experimental validation, we identified *CCNB1* as a vital gene in the adipogenesis of ADSCs. The expression of the *CCNB1* gene in adipocytes may contribute to an understanding of the biological characteristics of fat cells and the regulation of *CCNB1* during the fat transplantation process could be manipulated to enhance the success rate of fat grafting. We believe our findings represent new insights useful for further investigating the mechanisms of ADSCs in adipogenesis and their clinical utilization as stem cells.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (protocol code: 2022-NT-79). Informed consent was obtained from all participants involved in the study.

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