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PAMM Does not Affect Adipogenic Differentiation of Human Adipose-derived Stem Cells*

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Abstract **Objective** PAMM (peroxiredoxin-like 2 activated in M-CSF stimulated monocytes) is a secreted protein which shows high expression in white adipose tissues, but the roles of PAMM in many biological processes are still unknown. To provide new clues for PAMM function research, this study is intended to investigate the possible role of PAMM in white adipogenesis as well as the downstream genes regulated by PAMM. **Methods** Adipogenic differentiation and adipogenic inhibition models of human ADSCs (adipose-derived stem cells) were established using adipogenic cocktail (AC) medium or AC plus IL-1α, respectively. Expression of PAMM in ADSCs was suppressed or overexpressed using siRNA interference or plasmid transfection. Gene array, mRNA sequencing and quantitative RT-PCR were employed to detect the mRNA expression level. Western blot was used to evaluate protein expression and Oil red O staining was adopted to assess lipid droplets accumulation. **Results** Expression of PAMM was increased following white adipogenic differentiation of ADSCs and decreased following adipogenic inhibition. However, when PAMM was knocked down or overexpressed before adipogenic differentiation of ADSCs, the downregulation and upregulation of PAMM expression generally did not exert evident influence on the formation of lipid droplets and the expression of adipogenesis-related genes. Similarly, PAMM knockdown in highly differentiated adipocytes had no obvious effect on cellular morphology and the accumulation of lipid droplets. Finally, a bunch of functional genes and gene sets regulated by PAMM, such as *SULF1*, *A2M* genes and P53 stability gene set, were screened out and confirmed through siRNA interference, mRNA sequencing and qRT-PCR. **Conclusion** This study suggests PAMM could serve as a useful marker of white adipogenic differentiation of ADSCs, but it exerts no evident effect on white adipogenic differentiation. The unveiled downstream genes and gene sets regulated by PAMM would provide new clues for the functional research of PAMM.

Key words PAMM, adipocytes, adipogenic differentiation, stem cells, adipokines
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White adipose tissue plays important role in energy storage, energy generation and other physiological or metabolic processes[1]. White adipose tissue can also secrete a variety of bioactive peptides such as adiponectin, leptin and TNF-α, which are collectively known as adipokines[2]. These adipokines play essential roles in some pathological and physiological processes through the way of endocrine, paracrine or autocrine. Some metabolism-related diseases such as diabetes and obesity are closely related to dysfunctional expression of adipokines[3-4]. Therefore, exploring new adipokines and revealing their function would provide valuable information for disease prevention and therapy.

Adipose tissue keeps on self-renewal throughout lifespan. Under physiological conditions, about 10% of adipocytes are renewed every year[5]. ADSCs (adipose-derived stem cells) are mesenchymal stem cells isolated from adipose tissue with the potential of differentiating into adipocytes, osteoblasts and chondrocytes[6]. As seeding cells in tissue engineering, ADSCs have many advantages, including easy availability, high proliferation ability, and fewer ethical concerns. Differentiation of ADSCs into mature adipocytes is a complex multi-step process, involving in the up-regulation and down-regulation of many genes. We have established *in vitro* culture of human ADSCs which can proliferate and survive for long time in B27-containing medium without losing the differentiation potential[7].

PAMM, also known as FAM213A, adiporedoxin or peroxiredoxin-like 2A, is encoded by *PRXL2A* gene[8-15]. PAMM contains a CXXC motif which plays an important role in redox regulation[14-15]. Though comprehensively expressed in many tissues such as aorta, brain and testis, PAMM displays the highest expression level in adipose tissue[13]. It has been reported that PAMM could be secreted into extracellular space, possesses the function of anti-oxidation, anti-inflammation and plays important role in osteoclast differentiation and endothelial activation[11, 13-15]. However, most of the results of PAMM came from mouse tissue or cells. Our preliminary results from gene array revealed that the expression level of PAMM increased with white adipogenic differentiation and decreased with adipogenic inhibition of human ADSCs, which implied some functional roles of PAMM in adipose tissue. To explore the possible new function of PAMM, we first confirmed the correlation between PAMM expression level and adipogenic differentiation and then explored the possible role of PAMM in adipogenic differentiation of ADSCs. Finally, the downstream genes and gene sets regulated by PAMM were screened and confirmed by mRNA sequencing and qRT-PCR. This study would provide new clues for functional research of PAMM.

1 Materials and methods

1.1 Cell culture

The primary ADSCs from abdominal subcutaneous fat of a 35-year-old male were purchased from Dongguan Cell Biotechnology Co., Ltd. (Dongguan, China). The ADSCs were cultured as previously described[7] with low glucose medium containing Dulbecco's Modified Eagle Medium (DMEM, Gibco, CA, USA), 10% fetal bovine serum (FBS, Hyclone, USA) and 2% B27 supplement (Gibco) in a 5% CO_2, 37° C humidified atmosphere, and the medium was changed every 3 d. When the cells grew up to 80%−90% confluence, they were routinely digested with trypsin (Gibco) and passaged.

1.2 White adipogenic differentiation of ADSCs *in vitro*

To prepare adipogenic differentiation, ADSCs were seeded into a 12-well plate. When the cells became about 90%−100% confluence after 24−48 h, the grow medium was changed into adipogenic cocktail (AC) medium composed of DMEM, 10% FBS, 1 μmol/L of dexamethasone (D4902, Sigma, MO, USA), 10 mg/L of insulin (I8040, Solarbio Science & Technology Co., Ltd., China), 0.5 mmol/L of 3-isobutyl-1-methylxanthine (I5879, Sigma) and 100 μmol/L of indomethacin (I7378, Sigma). The AC medium was refreshed every 3 or 4 d when needed. Oil red O (O1391, Sigma) staining was performed to detect lipid droplet accumulation at day 7 after adipogenic differentiation.

1.3 Gene expression array

Three groups of cells were used for gene array, those are, ADSCs before differentiation, ADSCs undergone 3-day adipogenic differentiation, ADSCs undergone 3-day adipogenic differentiation together

with IL-1α (1 μg/L). The cells were collected for RNA extraction and the array detection was performed using the Agilent human lncRNA+mRNA Array V4.0 (USA). The data were analyzed for summarization, normalization and quality control using the GeneSpring software V13.0 (Agilent). Differential genes with a fold change more than 2 times and a *t*-test $P<0.05$ were collected for further analysis.

1.4 RNA interference

ADSCs or differentiated adipocytes derived from ADSCs were prepared for RNA interference. Human PAMM small interfering RNA (siRNA), a non-specific control siRNA (scrambled siRNA) and Lipofectamine™ RNAiMAX transfection reagent (13778150, Invitrogen) were diluted in Opti-MEM reduced serum medium (Gibco). The Opti-MEM medium was 200 μl/well, Lipofectamine™ RNAiMAX reagent was 2.5 μl/well, and the final concentration of siRNA was 50 nmol/L. The mixture was added into the culture wells after 15-minute incubation at room temperature. The inhibition efficiency of PAMM was detected by qRT-PCR or Western blot.

1.5 PAMM plasmid transfection

PAMM-related expression plasmids were kindly provided by Professor FU Min-Gui in the University of Missouri Kansas City[13]. The empty plasmid pCMV-MAT-Tag-FLAG-1 (Sigma) was used to construct the Flag-tagged human PAMM expression plasmid (Flag-PAMM plasmid). ADSCs were seeded into 12-well plates for either empty plasmid or PAMM plasmid transfection. Upon arriving 80% confluence of ADSCs, plasmids were transfected using FuGENE® HD transfection reagent according to the manufacturer's instructions.

1.6 Oil red O staining

After 7 d of adipogenic differentiation, cells were washed with PBS, fixed with 4% paraformaldehyde for 30 min and rinsed with 60% isopropyl alcohol. After rinsing, the cells were stained with Oil Red O solution for 30 min. The stained cells were washed 3 times with PBS, observed and taken images under a microscope. For quantification of the stained area, stained cells were eluted with 100% isopropyl alcohol and measured by absorbance value at 520 nm against a blank well (100% isopropyl alcohol) using a microplate reader.

1.7 RNA isolation, reverse transcription and quantitative PCR (RT−PCR)

Total RNA was extracted from cells with TRIzol® Reagent (Life Technologies, USA), according to the manufacturer's instructions. The cDNA was synthesized by reverse transcription using PrimeScript™ RT reagent kit (RR047A, TaKaRa, Japan). The PCR reaction was conducted on a LightCycler® 96 System (Roche, USA) with SYBR™ Select Master Mix (4472908, Applied Biosystems, USA), and the reaction system was 10 μl. The primers sequences were showed in Table 1. GAPDH served as an internal reference gene.

Table 1 The primer sequences in this study

Gene	Forward	Reverse	Product/bp
PAMM	5'-ATGGGGATGTGGTCCATTGG-3'	5'-GCACGGCCATAATCACAGC-3'	208
PPARγ	5'-TACTGTCGGTTTCAGAAATGCC-3'	5'-GTCAGCGGACTCTGGATTCAG-3'	141
C/EBPα	5'-ACTTGGTGCGTCTAAGATGAGGG-3'	5'-GCATTGGAGCGGTGAGTTTG-3'	145
Adiponectin	5'-GGCTTTCCGGGAATCCAAGG-3'	5'-TGGGGATAGTAACGTAAGTCTCC-3'	103
FABP4	5'-ACTGGGCCAGGAATTTGACG-3'	5'-CTCGTGGAAGTGACGCCTT-3'	183
GAPDH	5'-ACAACTTTGGTATCGTGGAAGG-3'	5'-GCCATCACGCCACAGTTTC-3'	101

1.8 Western blot

Cells were collected, washed with PBS, and lysed in RIPA buffer containing 50 mmol/L Tris (pH 7.4), 150 mmol/L NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and other inhibitors such as sodium orthovanadate, sodium fluoride, EDTA and

leupeptin. Equal amounts of protein samples were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis, and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, MA, USA). The membrane was then blocked with 5% milk solution and followed by incubation with primary antibodies overnight at 4℃. Subsequently, the membranes were washed 3 times with TBS containing 0.05% Tween 20 (TBST). After incubation with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature, the membrane was exposed using the Azure c400 imaging system (Azure Biosystems, Inc., USA).

The following antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA): peroxisome proliferators-activated receptors gamma (PPARγ) (#2443) and CCAAT/enhancer binding protein (C/EBPα) (#8178). Adiponectin (21613-1-AP) and fatty acid-binding protein 4 (FABP4) (12802-1-AP) antibodies were obtained from Proteintech Group, Inc. (Rosemont, IL, USA). Anti-PAMM (HPA009025) was purchased from Sigma-Aldrich (USA). Anti-Tubulin (A11126) was obtained from Invitrogen (Carlsbad, CA, USA).

1.9 Sequencing of mRNA

PAMM-targeting siRNA was used to suppress PAMM expression. Four groups of cells, including ADSCs before differentiation (NC), ADSCs induced into adipogenic differentiation after transfection with control siRNA (Adi + Scrambled), PAMM si-1 (Adi + si-1) and PAMM si-3 (Adi + si-3) siRNA, were used for mRNA sequencing. BGISEQ-500 platform (www.genomics.org.cn, BGI, Shenzhen, China) was used. Genes expression levels were quantified by a software package RSEM[16]. DEGseq method was used to screen differentially expressed genes (DEGs) between different groups[17]. GSEA (gene set enrichment analysis) was performed to pool the functional gene sets regulated by PAMM with GSEA software (Broad Institute, Inc. USA). Metric for ranking genes adopted “Ratio of Classes”. The results with |NES|>1, P<0.05 and FDR< 0.25 were regarded as significant.

1.10 Statistical analysis

The data were analyzed using SPSS 19.0 software. The experimental data were expressed as mean±SD, and the difference of mean was evaluated by student’s t test or an analysis of variance. P<0.05 was considered to be significant.

2 Results

2.1 Expression of PAMM is closely related to white adipogenic differentiation of ADSCs

The ADSCs were cultured as previously reported, which expressed specific markers of adipose stem cells and were multipotent[7]. The AC medium was used to induce ADSCs to differentiate towards mature adipocytes. After 3-day induction, the cells morphology changed from long spindle to oval, with tiny lipid droplets in cytoplasm. When prolonging the induction up to 7 d, massive lipid droplets could be observed in the cytoplasm of the differentiated adipocytes.

To explore potential adipogenesis-related genes, adipogenic differentiation and adipogenic inhibition of ADSCs were established by utilizing AC medium or AC medium plus the cytokine IL-1α (Figure 1a−c), which strongly inhibited adipogenesis[7]. Then the differential gene expression was investigated by gene array in ADSCs before differentiation, 3-day adipogenic differentiation or 3-day differentiation plus IL-1α. From the array data, 770 adipogenesis-related genes were screened out which were up-regulated in adipogenic differentiation and down-regulated in adipogenic inhibition conditions (Figure 1d). These genes included the classical adipogenesis-regulating genes, such as peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer binding protein alpha (C/EBPα), which suggested that the strategy was effective in screening adipogenesis-regulating genes. From the 770 genes, we selected 10 genes including PAMM for further verification by qRT-PCR. The results showed that expression of PAMM increased dramatically with adipogenic differentiation and decreased significantly with adipogenic inhibition (Figure 1e). Meanwhile, PAMM displayed high abundance relative to other genes (data not shown). Therefore, PAMM was selected for further research.

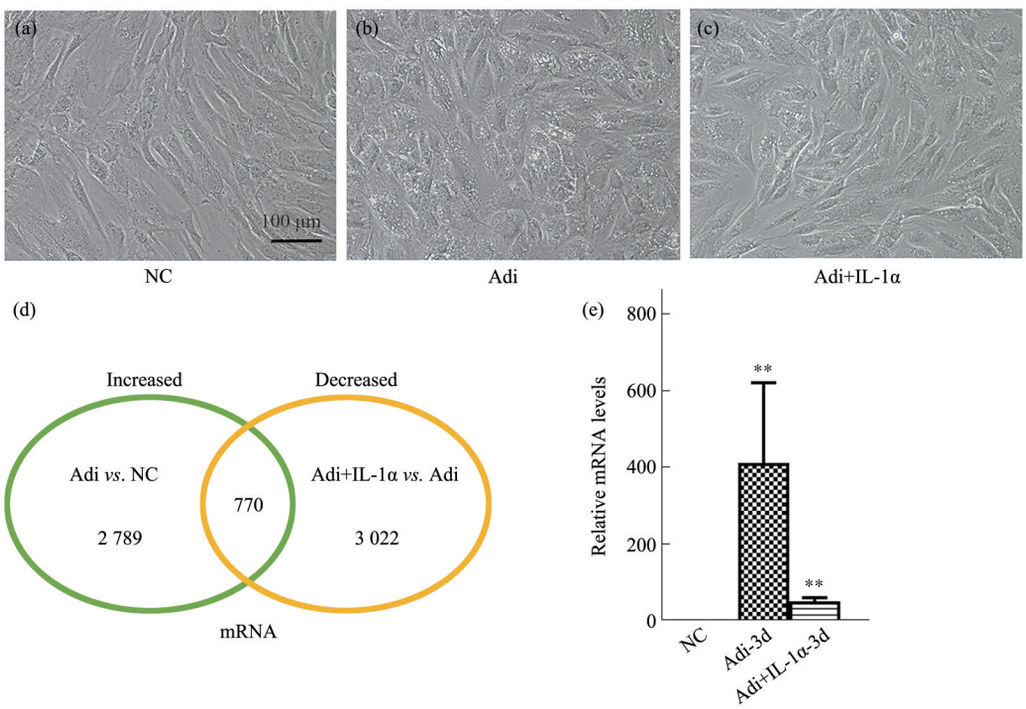

Fig. 1 Screening out potential adipogenesis−related genes based on adipogenic differentiation and inhibition

Adipogenic differentiation or inhibition was established by 3-day incubation in adipogenic medium (Adi) or Adi plus IL-1α. Gene array was employed to detect mRNA expression. (a−c) Cellular morphology of ADSCs in undifferentiation (NC), adipogenic differentiation or adipogenic inhibition (Adi+IL-1α) conditions. (d) Enrichment of potential adipogenesis-related genes using Venn diagram. The genes which concurrently displayed increase in Adi/NC (Adi *vs.* NC, green circle) and decrease in Adi+IL-1α/Adi (Adi+IL-1α *vs.* Adi, yellow circle) were pooled as possible adipogenesis-related genes. (e) Expression change of PAMM in adipogenic differentiation and inhibition conditions, evaluated by qRT-PCR. ***P*< 0.01 *vs.* NC; ##*P*<0.01 *vs.* Adi (*n*=3).

2.2 PAMM knockdown has no significant effect on adipogenic differentiation

To investigate the possible role of PAMM in adipogenic differentiation, we used siRNAs to interfere PAMM expression in ADSCs or the differentiated adipocytes. ADSCs were transfected with siRNA for 24−48 h and then adipogenic differentiation were induced for 3−4 d. qRT-PCR and Western blot demonstrated that PAMM and adipocyte specific genes, including PPARγ, C/EBPα, adiponectin and fatty acid-binding protein (FABP4), were evidently upregulated after adipogenic induction (Figure 2a−f). After knocking down of PAMM by si-1 and si-3 (Figure 2a, f), qRT-PCR showed that expression of adipogenesis-related genes including PPARγ, C/EBPα, FABP4 and adiponectin decreased slightly, but the differences were not significant (Figure 2b−e). Western blot demonstrated that although PAMM knockdown induced weak decrease in the expression of the adipokines adiponectin and FABP4, it exerted no effect on C/EBPα expression (Figure 2f). When prolonging the adipogenic induction up to 7 d, Oil red O staining showed that silencing PAMM by either si-1 or si-3 had no obvious influence on lipid droplet accumulation (Figure 2g−l). The combined results suggested that PAMM knockdown had no evident effect on adipogenesis.

To further confirm whether PAMM plays a role in maintaining the mature status of adipocytes, PAMM knockdown was performed in the highly differentiated adipocytes derived from ADSCs. qRT-PCR and Western blot showed that inhibiting PAMM expression exerted no obvious effect on the detected adipogenesis- or adipocyte-related genes (Figure 3a−f). Oil red O staining demonstrated that down-regulation of PAMM had no evident effect on either cellular morphology or lipid droplets accumulation of the differentiated adipocytes (Figure 3g−l). The results

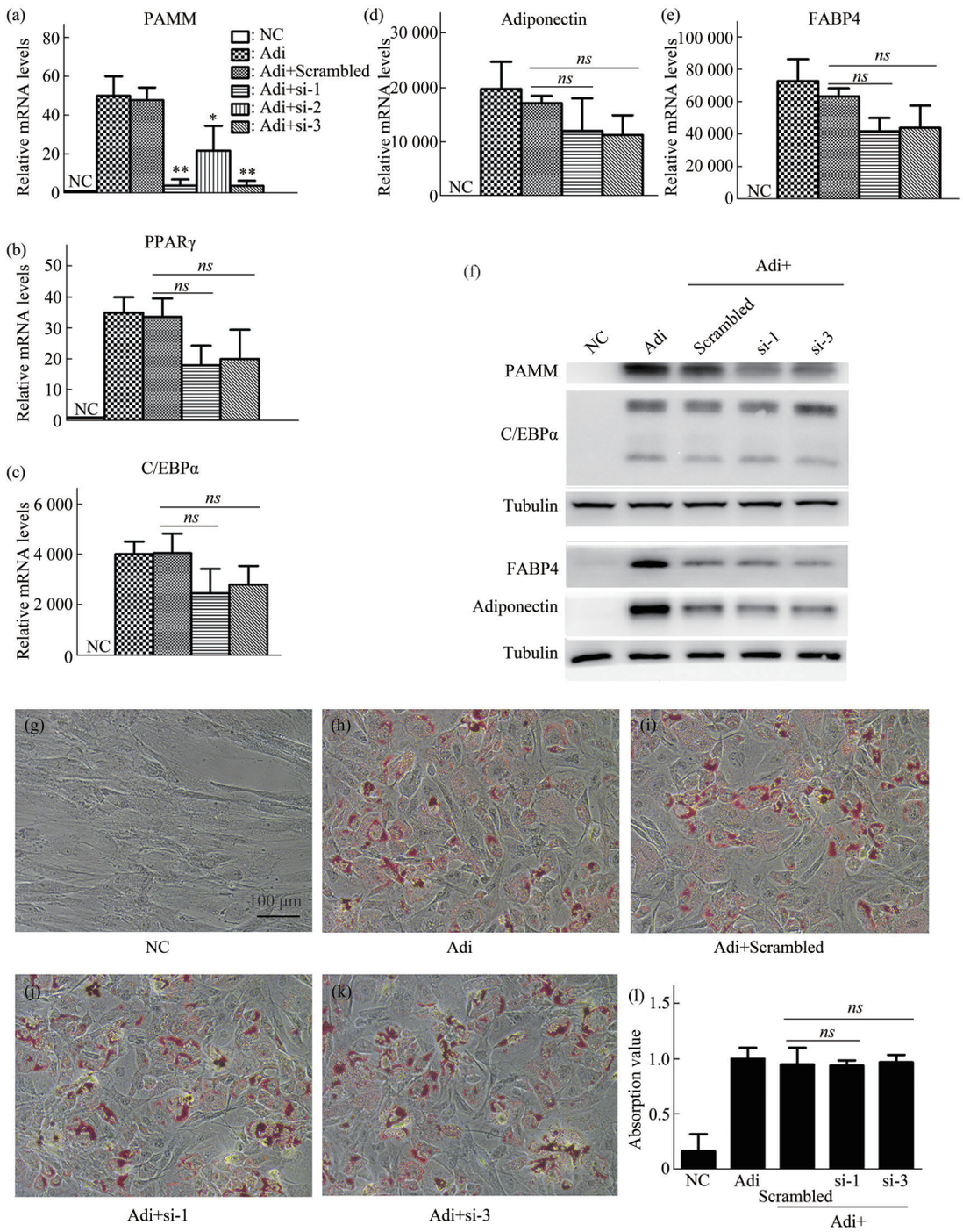

Fig. 2　PAMM knockdown has no significant effect on adipogenic differentiation of ADSCs

ADSCs were transfected with PAMM-targeting siRNA (si-1, si-2 or si-3) for 24−48 h and then induced toward adipogenic differentiation (Adi) for 3 or 7 d. NC is normal ADSCs. Scrambled siRNA acted as control. (a) Screening of effective siRNA molecular which could suppress PAMM expression by qRT-PCR. (b−f) The effect of PAMM knockdown on the expression of adipogenesis-related genes, including PPARγ, C/EBPα, adiponectin and FABP4, in mRNA (b−e) and protein (f) levels, after 3-day adipogenic differentiation. (g−l) Prolonging adipogenic differentiation up to 7 d after siRNA interference and then Oil red O staining was performed. (g−k) The representative pictures of Oil red O staining. (l) Statistical absorbance values from 3 independent experiments. **P*<0.05, ***P*<0.01 *vs.* Adi+Scrambled (n=3); *ns* means not significant (*n*=3).

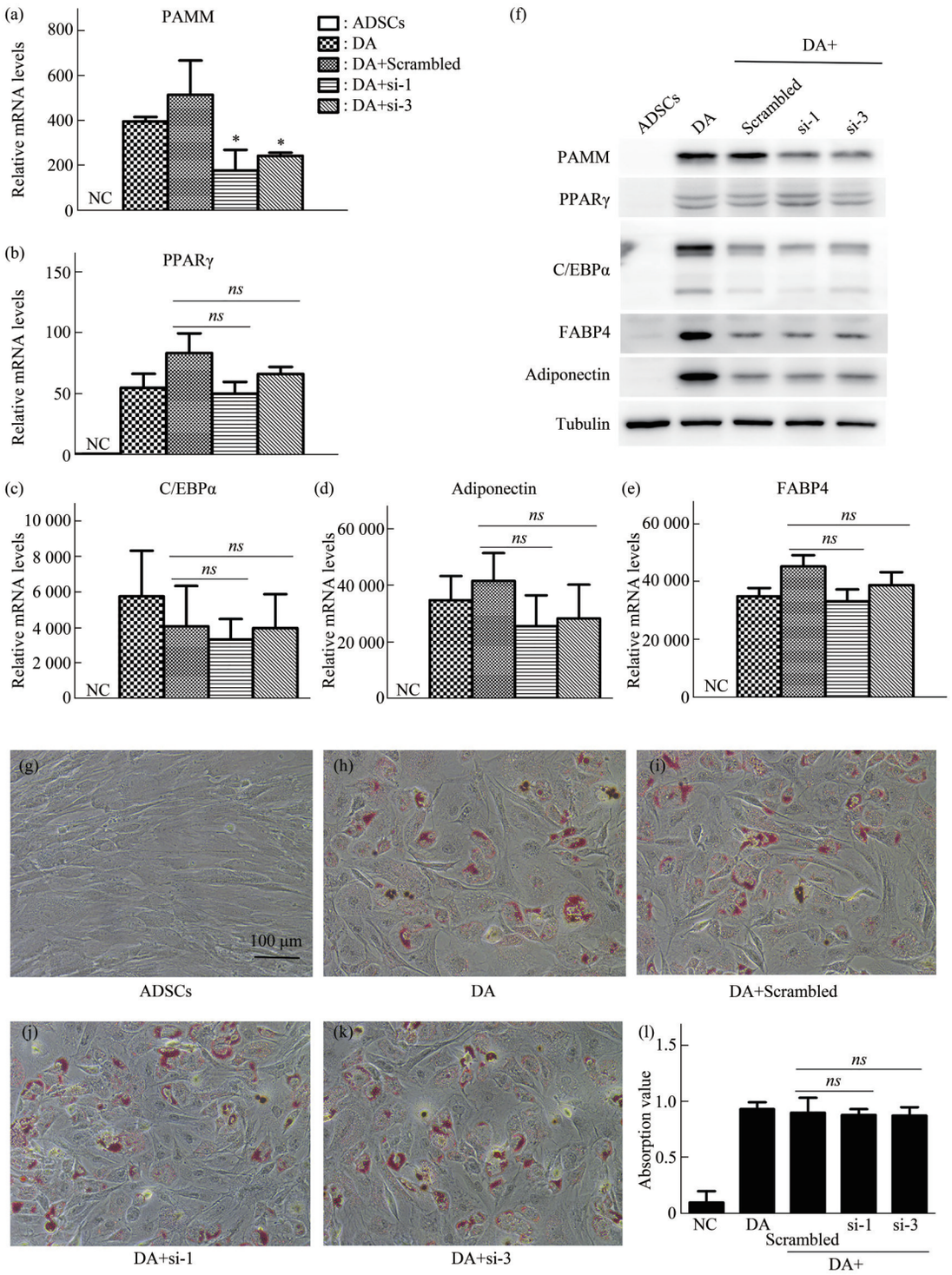

Fig. 3　PAMM knockdown in highly differentiated adipocytes exerts no significant effect on adipogenesis−related genes and the accumulation of lipid droplets

ADSCs were firstly induced into highly differentiated adipocytes after 7-day induction and then PAMM was knocked down by siRNA transfection. (a) Verification of PAMM knockdown in differentiated adipocytes (DA). Expression of PAMM was detected by qRT-PCR in ADSCs (NC), DA and DA transfected with scrambled or PAMM-targeting (si-1 and si-3) siRNA. (b−f) The expression of adipogenesis-related genes in mRNA (b−e) and protein (f) levels after PAMM knockdown in differentiated adipocytes. (g−k) Oil red O staining of the differentiated adipocytes after PAMM knockdown. (l) Statistical absorbance values of Oil red O staining. **P*<0.05 *vs.* DA+Scrambled (*n*=3); *ns* means not significant (*n*=3).

further implied that PAMM was dispensable for adipogenesis.

2.3 Overexpression of PAMM in ADSCs has no significant effect on adipogenic differentiation

To further verify the conclusion in siRNA interference, we investigated the effect of PAMM overexpression on adipogenic differentiation. The Flag-PAMM plasmids were transfected into ADSCs and the overexpression was confirmed by qRT-PCR and Western blot (Figure 4a, b). Then, the transfected cells were induced towards adipogenic differentiation for 7 d. Similar change of cellular morphology was observed in both groups. Oil red O staining demonstrated that there was no significant difference in the amount and distribution of lipid droplets between Flag-PAMM plasmid and empty plasmid (Figure 4c−g).

It has been reported that PAMM is a secretory protein[13]. We also overexpressed PAMM in human 293T cells and collected cell supernatant and the whole cell lysate for Western blot analysis. The results showed that PAMM could be detected in culture supernatant (Figure 4h), which was consistent with the previous report[13].

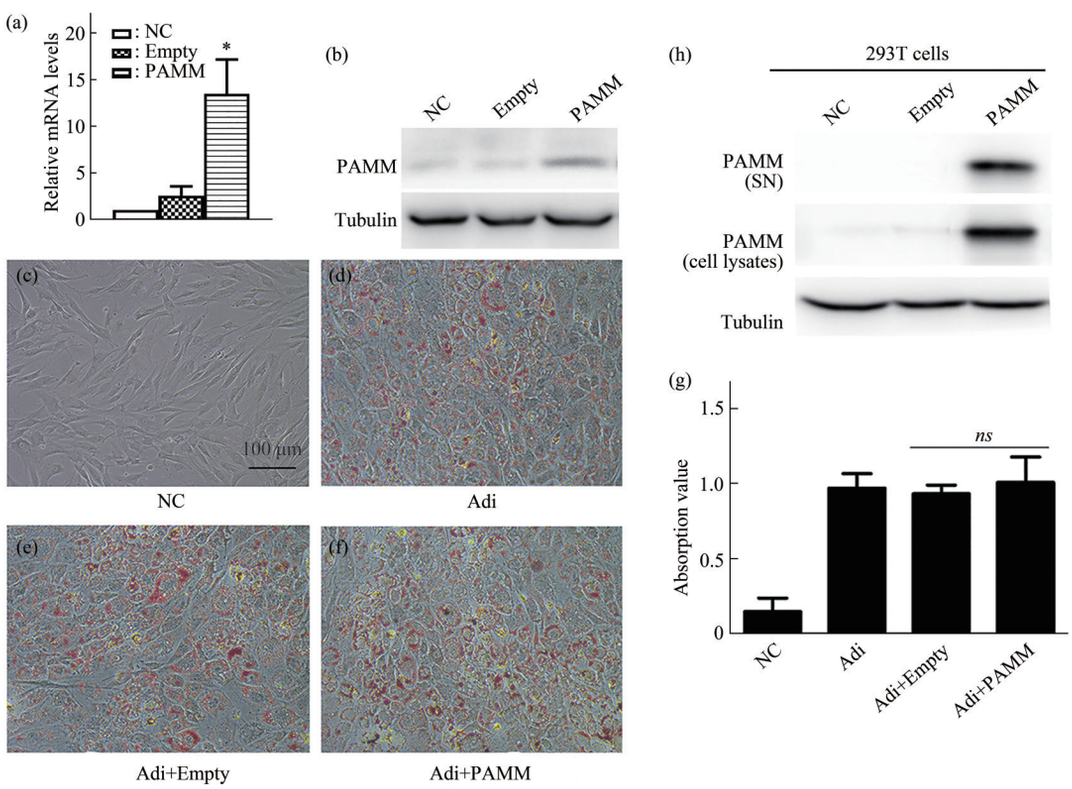

Fig. 4 Overexpression of PAMM in ADSCs does not significantly affect adipogenic differentiation

(a−g) ADSCs were transfected using PAMM or empty plasmids (a, b) and then induced towards adipogenic differentiation for 7 d (c−g). The overexpression of PAMM was evaluated by qRT-PCR (a) and Western blot (b). (c−g) Oil red O staining was performed after adipogenic differentiation and then absorbance values were detected (g). NC is normal ADSCs before differentiation. (h) Human embryonic kidney cells (293T) were transfected with empty or PAMM plasmid and then the cell supernatant (SN) and whole cell lysates were collected for Western blot. *P<0.05 *vs.* Empty (n=3); *ns* means not significant (n=3).

2.4 Exploring the downstream genes regulated by PAMM

To provide new clues for functional research of PAMM, the specific genes regulated by PAMM in adipocytes were investigated. After suppressing PAMM expression with specific siRNA fragment (si-1 or si-3), adipogenic differentiation of ADSCs for 3 d was adopted and then mRNA sequencing was

performed. The differential genes with ≥2 fold change between scrambled and si-1 or si-3 siRNA were harvested for further analysis.

The results showed that, compared with scrambled siRNA, 138 genes were up-regulated and 148 genes were down-regulated in si-1 group, while 128 genes were up-regulated and 118 genes were down-regulated in si-2 group (Figure 5a, b). Venn analysis showed that 20 genes were concurrently upregulated while 29 genes were concurrently downregulated (including PAMM) by both si-1 and si-3 fragments (Figure 5c, d). Then the expression levels of 20 up-regulated and 29 down-regulated genes were further verified by qRT-PCR. Among which, *ZBED5-AS1*, *SULF1* and *A2M* were evidently increased, and *FAIM2*, *CITED1* and *HSD11B1* were significantly decreased after PAMM knocking down (Figure 5e−g). The results suggested that PAMM positively regulated the expression of FAIM2, CITED1 and HSD11B1, and negatively regulated the expression of ZBED5-AS1, SULF1 and A2M.

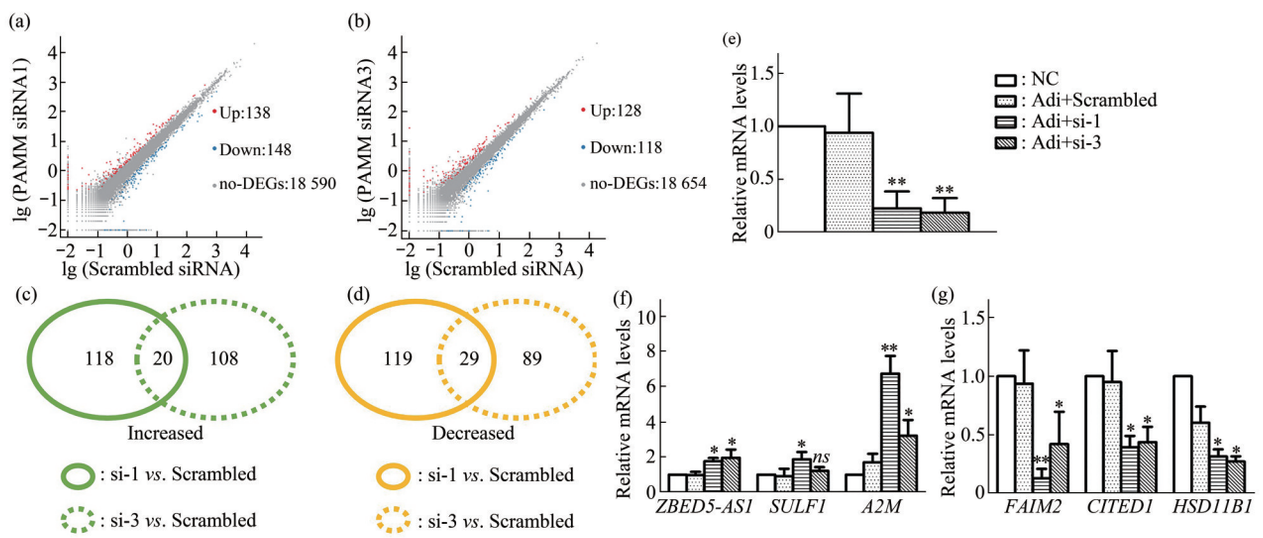

Fig. 5 Screening out the genes regulated by PAMM during adipogenic differentiation of ADSCs

ADSCs were induced towards adipogenic differentiation for 3 d after PAMM knockdown and then mRNA sequencing was performed. (a, b) Differential expression genes after PAMM knockdown by si-1 or si-3. The red spots represent upregulated genes and the green spots represent downregulated genes *vs.* scrambled siRNA. (c, d) Venn diagram of the differential expression genes. The genes which were consistently upregulated (green circle) or downregulated (yellow circle) by both si-1 and si-3 were pooled. (e−g) Verification of the differential genes screened out from mRNA sequencing by qRT-PCR. After PAMM knockdown by si-1 and si-3 (e), some genes displayed increased expression (f) and some others displayed decreased expression (g). **P*<0.05 and ***P*<0.01 *vs.* Adi+Scrambled; *ns* means not significant *vs.* Adi+Scrambled (*n*=3).

Since the differential genes concurrently regulated by si-1 and si-3 siRNA were relative less, GSEA analysis was performed to explore functional gene sets regulated by PAMM siRNA, which was based on the expression levels of all genes. The results showed that some biological pathways or functions were synchronously downregulated by both si-1 and si-3 siRNA, compared with scrambled siRNA, which included Runx2 regulating bone development, regulation of Runx3 expression and activity, stabilization of P53, downstream signaling events of β cell receptor, and among others (Figure 6). The results implied functional roles of PAMM in these biological processes.

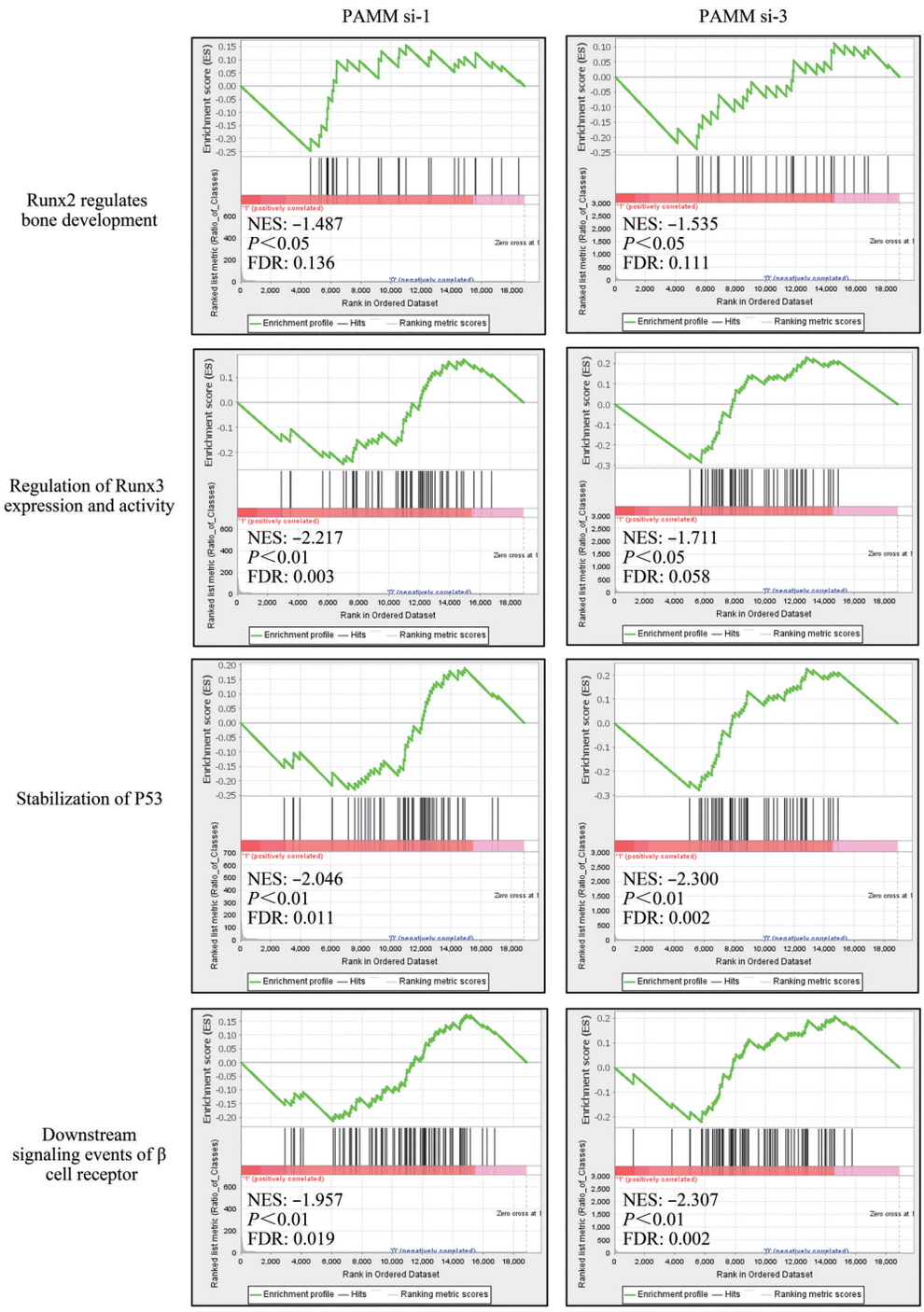

Fig. 6　Effect of PAMM knockdown on biological gene sets of differentiated ADSCs evaluated by GSEA analysis

The mRNA-seq data after PAMM knockdown were analyzed with GSEA software. The functional gene sets downregulated by both si-1 and si-3 siRNA, compared with scrambled siRNA, were obtained.

3　Discussion

White adipose tissue is one of the most widely distributed tissues in the human body. Adipogenesis is regulated by a series of transcription factors and various signaling pathways, such as PI3K/AKT, Nrf2, Wnt/β-Catenin, *etc*.[18-20]. Among these transcription factors, PPARγ and C/EBPα are the master genes regulating adipogenesis. PPARγ is a nuclear hormone

receptor, which is highly expressed in mammalian adipose tissue. PPARγ alone can initiate the adipogenic differentiation process[21]. C/EBPα is an important member of the C/EBPs family and could be activated by PPARγ to regulate adipogenic differentiation[22]. Dexamethasone, one of the components in adipogenic cocktail medium, can upregulate the expression of PPARγ and C/EBPα to promote adipogenesis[23]. FABP4 is mainly found in adipose tissue and macrophages, and plays an important role in regulating metabolism and inflammation[24]. Adiponectin, an adipokine abundantly secreted by adipocytes, has anti-diabetic, anti-atherosclerotic and anti-inflammatory properties[25]. Adiponectin has become a marker gene of adipogenic differentiatio[26].

In 2010, Xu *et al*.[14] reported that PAMM is a redox regulatory protein that regulates osteoclast differentiation *in vitro*. Expression of PAMM contributes to bone mass maintenance. As an antioxidant protein, the expression level of PAMM is mediated by Akt phosphorylation[11]. Meanwhile, studies by Guo *et al*.[13] found that PAMM can inhibit macrophage inflammation by inhibiting the MAPK signaling pathway, and this anti-inflammatory effect is not achieved by its antioxidant effec. PAMM also plays a key role in metabolism regulation and endothelial cell activation by regulating protein secretion and inhibiting MAPK and NF-κB signaling[12, 15]. Recently, it was reported that PAMM could act as a marker of acute spinal cord injury[10] as well as a novel prognostic factor of acute myeloid leukemia[9]. The functional variety of PAMM demonstrates that it is an important protein involved into many physiological and pathological processes.

Since PAMM displays high expression in white adipose tissue, it would be interesting to explore if PAMM plays a role in white adipogenesis. In this study, we found that PAMM was upregulated with adipogenic differentiation and downregulated with adipogenic inhibition. However, knockdown or overexpression of PAMM in human ADSCs did not significantly influence adipogenic differentiation, manifested by change of cellular morphology, accumulation of lipid droplets and the expression of adipogenesis master genes. Similarly, knockdown of PAMM in highly differentiated adipocytes did not influence cellular morphology and the accumulation of lipid droplet. These results suggest that, though the expression level of PAMM is positively correlated with adipogenesis, it exerts no evident effect on adipogenic differentiation. Meanwhile, human PAMM can be secreted into extracellular space when overexpressed in 293T cells, which is consistent with the extracellular location of adipokine.

Current study further screened and verified a bunch of genes regulated by PAMM, which would provide new clues for PAMM functional research. Among which, *SULF1* encodes extracellular heparan sulfate sulfatase, which is down-regulated in many types of cancer and participates into drug resistance[27]. SULF1 also responds to exogenous stimuli to inhibit heparin-dependent growth factor signals and reduce proliferation[28]. A2M mediates the clearance and degradation of Aβ (the major component of β-amyloid deposition) protein and is associated with Alzheimer's disease[29]. *HSD11B1* encodes a protein that catalyzes the conversion of cortisone to cortisol, which could lead to central obesity. A particular variant of HSD11B1 is associated with childhood obesity and insulin resistance[30]. Therefore, PAMM might exert its functional role by regulating these downstream genes, which need to be explored further.

GEAS analysis obtained a bunch of functional gene sets regulated by PAMM. Among which, *Runx2* and *Runx3* are related to osteoblast and chondrocyte differentiation[31-32]. Combined with the reported function of PAMM in blocking osteoclast differentiation[14], the study implies an important role of PAMM in maintaining bone mass. Regulation of the P53 protein (a key tumor suppressor) stabilization and β cell signal pathway by PAMM implies a possible role of PAMM in cancer and diabetes. Further investigation would be necessary to confirm these possible functions of PAMM.

4 Conclusion

Current study suggests that PAMM expression is the concomitant result of white adipogenic differentiation of ADSCs and could act as a marker of white adipogenesis. PAMM doesn't exert evident effect on adipogenesis but might play a regulatory role in other biological functions by regulating the expression of *SULF1*, *A2M* and *HSD11B1* gene, among others.

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PAMM不影响人脂肪干细胞的成脂分化能力*

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摘要　目的　PAMM（peroxiredoxin-like 2 activated in M-CSF stimulated monocytes）是近年来发现的一种在脂肪组织中高表达的分泌因子，但其生物学功能尚不完全清楚。为了给PAMM功能研究提供新线索，本文探讨了PAMM在白色脂肪生成中的可能作用及PAMM调节的下游基因。**方法**　利用成脂分化液或“成脂分化液+IL-1α”建立人脂肪干细胞（adipose-derived stem cells，ADSCs）的成脂分化和成脂抑制模型。用siRNA干扰和过表达质粒转染的方法抑制或上调PAMM表达水平。采用基因芯片、mRNA测序及定量RT-PCR检测基因的mRNA表达水平，采用蛋白质印迹法评价蛋白质表达水平，用油红O染色检测脂滴沉积。**结果**　随着ADSCs向白色脂肪分化，PAMM表达水平明显上升，而随着成脂抑制，PAMM表达明显下降。在ADSCs中沉默或过表达PAMM后，再进行成脂分化。结果发现，上调或下调PAMM对脂滴形成及成脂相关基因的表达均无明显影响。类似地，在高度分化的脂肪细胞中敲低PAMM表达，对细胞形态及脂滴含量亦无明显影响。本文进一步利用siRNA干扰、mRNA测序及qRT-PCR，筛选和验证了一批受PAMM调节的下游基因及功能性基因集，包括*SULF1*、*A2M*基因及调节P53稳定性的基因集等。**结论**　PAMM可作为白色成脂分化的标志基因，但其自身对白色脂肪细胞生成无明显影响，本研究筛选出来的PAMM下游基因及基因集，可为进一步研究PAMM的功能提供新线索。

关键词　PAMM，脂肪细胞，成脂分化，干细胞，脂肪因子
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