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Leukemia cells remodel marrow adipocytes via TRPV4-dependent lipolysis

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Authors’ contributions

S.Y. and W.L. contributed equally to this work. S.Y. performed the experiments, analyzed the data and wrote the manuscript; W.L. performed the experiments; C. Z. and Y. Z. collected patient samples; Y.W. and J.L. isolated the MSCs; Y.Y. performed the immunohistochemistry; Z.L. analyzed the results and revised the paper; J.S. conceived the overall study, designed the experiments and revised the paper. All authors read and approved the final version of the submitted manuscript.

Running heads: TRPV4 mediates marrow adipocyte remodeling in AML

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Remodeling of adipocyte morphology and function plays a critical role in prostate cancer development. We previously reported that leukemia cells secrete growth differentiation factor 15 (GDF15), which remodels the residual bone marrow (BM) adipocytes into small adipocytes and is associated with a poor prognosis in acute myeloid leukemia (AML) patients. However, little is known about how GDF15 drives BM adipocyte remodeling. In this study, we examined the role of the transient receptor potential vanilloid (TRPV) channels in the remodeling of BM adipocytes exposed to GDF15. We found that TRPV4 negatively regulated GDF15-induced remodeling of BM adipocytes. Furthermore, transforming growth factor-β type II receptor (TGFβRII)
was identified as the main receptor for GDF15 on BM adipocytes. PI3K inhibitor treatment reduced GDF15-induced pAKT, identifying PI3K/AKT as the downstream stress response pathway. Subsequently, GDF15 reduced the expression of the transcription factor Forkhead box C1 (FOXC1) in BM adipocytes subjected to RNA-seq screening and Western blot analyse. Moreover, it was also confirmed that FOXC1 combined with the TRPV4 promoter by the Chip-qPCR experiments, which suggests that FOXC1 mediates GDF15 regulation of TRPV4. In addition, an AML mouse model exhibited smaller BM adipocytes, whereas the TRPV4 activator 4α-phorbol 12,13-didecanoate (4αPDD) partly rescued this process and increased survival. In conclusion, TRPV4 plays a critical role in BM adipocyte remodeling induced by leukemia cells, suggesting that targeting TRPV4 may constitute a novel strategy for AML therapy.

**Keywords:** Acute myeloid leukemia, GDF15, TRPV4, FOXC1, Bone marrow adipocyte

**Introduction**

The development of acute myeloid leukemia (AML) is closely related to the bone marrow (BM) microenvironment.\(^1\)\(^2\) As a critical component of the BM microenvironment, BM adipocytes provide energy for both the infinite proliferation of leukemia cells and the normal growth of hematopoietic stem cells.\(^3\)\(^4\) Leukemia cells proliferate to an overwhelming number in a limited marrow cavity, likely because these cells are more efficient in capturing energy for growth. Accordingly, BM adipocytes are remodeled in response to leukemia cells, generating a pro-tumoral
microenvironment.\textsuperscript{5,6} However, the mechanism whereby leukemia cell growth induces BM adipocyte remodeling is still unclear.

Induced BM adipocyte remodeling involves several specific processes, including lipolysis, dedifferentiation and lipid accumulation. Consequently, the remodeled adipocytes show morphological and functional changes.\textsuperscript{7} Breast cancer cells reportedly secrete soluble factor Wnt3a that reduces the number and size of adipocytes surrounding the malignant cells and thus contributes to disease development.\textsuperscript{8} As the breast cancer progresses, adipocytes dedifferentiate to fibroblast-like cells.\textsuperscript{8} In mouse models of bone metastasis following prostate cancer, Herroon et al. showed that remodeled BM adipocytes support tumor growth by FABP4 transportation of fatty acids.\textsuperscript{9} These studies identified a functional role of remodeled adipocytes in supporting solid tumor metabolism. Thus, the adipocytes remodeled by cancer cells are also known as cancer associated adipocytes.\textsuperscript{10} In the context of leukemia, there is a growing consensus that reduction in BM adipocyte number, once believed to be merely due to mechanical squeezing by the rapid proliferation of leukemia cells in the limited BM cavity, is also actively regulated by leukemia cells.\textsuperscript{5,11} Indeed, we previously reported that growth differentiation factor 15 (GDF15) derived from leukemia cells regulates BM adipocyte remodeling by enhancing lipolysis.\textsuperscript{12} However, how extracellular GDF15 induces lipolysis within BM adipocytes remains elusive.

It has been reported that GDF15 enhances intracellular Ca\textsuperscript{2+} by increasing calcium voltage-gated channel subunit alpha1 C (Cav1.3) expression in rat cerebellar granule neurons, which induces the expression of genes essential for synaptic plasticity.\textsuperscript{13} As an
important cellular signal for lipid metabolism, intracellular Ca^{2+} is involved in lipid synthesis and lipolysis in adipocytes.\textsuperscript{14,15} When the calcium channels in the adipocytes are activated or upregulated, accumulation of lipids is enhanced through increased [Ca^{2+}]_i.\textsuperscript{16,17} Conversely, when calcium channels are inhibited or downregulated, decreased calcium influx may accelerate fat breakdown.\textsuperscript{18,19} Thus, we hypothesized that calcium channels are involved in GDF15-induced BM adipocyte remodeling.

In this study, we examined a possible role of transient receptor potential vanilloid 4 (TRPV4) calcium channels in GDF15-driven remodeling of BM adipocytes. We unravel a novel function of TGF\textbeta RII that, in responding to GDF15 in BM adipocytes, activates the PI3K/AKT transduction pathway, which in turn reduces the transcript factor FOXC1 level and subsequently downregulates TRPV4. We also provide evidence that inhibition of BM adipocyte remodeling increases survival in the AML mouse model, implicating a novel therapeutic target for AML.

**Methods**

**Patients’ samples**

BM aspirates were collected from 16 patients diagnosed as lymphoma without BM invasion, using procedures approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Hospital. Mesenchymal stem cells (MSCs) were derived from the BM of lymphoma patients without BM invasion, because marrow MSCs in the type of patients can be considered normal.

**Chromatin immunoprecipitation-qPCR**
Adipocytes were collected from different groups and crosslinked with 1% formaldehyde for 10 min at 37°C. Cross-linking was blocked, then the cells were washed and lysed in SDS lysis buffer (50 mM HEPE NaOH 7.5, 500 mM NaCl, 1 mM EDTA, 0.1% Na-Deoxycholate, 1% Triton X100). The lysates were sonicated to shear DNA to a length between 200 and 500 base pairs with 10-second pulses using sonication. The antibody against Forkhead box C1 (FOXC1) (5 μL, Abcam5079, USA) was then added to the supernatant, incubated overnight at 4°C with rotation and incubated with 100 μL Salmon Sperm DNA/Protein A agarose beads for 2 h at 4°C. Then, the immunoprecipitated complex was washed and eluted. The histone DNA crosslinks were reversed and DNA was purified for real-time PCR. Quantitative real-time PCR was performed on bound and input DNA with the following primers for TRPV4 forward: 5-CTTTGCAGTGGGGAGCAGAGT-3, reverse: 5-ATTAACCG TGGGCTTCAGGCA-3.

Animal experiments

All animal experiments were performed according to procedures approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Hospital. Five-week-old C57BL/6 mice were fed with 60% high-fat diet (Research Diets, Inc. New Brunswick, NJ 08901, USA) for three months to construct an obese mouse model. Mice injected with FBL-3 cells (5×10^5) and mice injected with FBL-3 cells (5×10^5) and 4α-phorbol 12,13-didecanoate (4αPDD) (200 μg/Kg according to the instructions for reagents) were used as experimental groups. The untreated obese mice were used as a control group. The volume of all solutions injected was 200 μL. Mice were sacrificed and
femurs were removed after three weeks of treatment. Femurs were fixed for 24 h with 4% paraformaldehyde and were decalcified for two days. BM sections of mice were dewaxed by conventional methods. Anti-perilipin1 monoclonal antibody (1:50, CST, USA) was incubated at 4°C overnight.

**Statistical analysis**

All statistical tests were performed with GraphPad Primer5. The data were presented as the mean ± standard deviation (SD). Student’s t test was used to compare between two groups. A p value of less than 0.05 was considered statistically significant.

**Results**

**Downregulated TRPV4 contributes to increased bone marrow adipocyte lipolysis**

As an important channel for calcium ions, TRPV plays a critical role in the energy balance of adipocytes. RT-qPCR analysis showed that TRPV4 mRNA in BM adipocytes had the highest expression among TRPV family members (Figure 1A). Moreover, Western blot analysis showed that BM adipocytes highly expressed TRPV4 protein (Figure 1B). To address whether TRPV4 plays an important role in BM adipocytes, we used TRPV4 inhibitor (RN1734) and agonist (4αPDD) to verify the function of TRPV4 in BM adipocytes, respectively. 4αPDD is the first synthetic TRPV4 agonist and is a non-PKC activated phorbol ester. Supplementary Figure S1A showed the IC50 of the effects of RN1734 on BM adipocytes. Considering excessive Ca^{2+} influx could cause some toxicity to adipocytes, we aim to find a concentration that minimizes the cellular toxicity and promotes Ca^{2+} influx needed for our experiments. As Supplemental Figure S1B showed, 4αPDD at a concentration of 0.25 μg/mL resulted in an acceptable level
of toxicity of adipocytes, while allowing Ca\(^{2+}\) influx to reach the level required by the experiment. Oil red O (ORO) staining and quantitative analysis showed that RN1734 reduced the number and area of BM adipocytes, whereas \(4\alpha\)PDD did not induce a similar change (Figure 1C, D), suggesting that the inhibition of TRPV4 contributes to reducing BM adipocyte number and size. Furthermore, optical density (OD) value measurements showed that lipid droplets in BM adipocytes treated with RN1734 decreased significantly (Figure 1E).

In order to determine whether the phenomenon is related to lipolysis, we determined the rate-limiting enzymes (adipose triglyceride lipase, ATGL and hormone sensitive lipase, HSL) of lipolysis. \(^{22}\) ATGL catalyzes the first step of lipolysis and converts triglyceride to diacylglycerol and free fatty acids (FFAs). \(^{23}\) HSL is a hydrolase of glycerides and cholesterol esters. \(^{24}\) Along with TRPV4 channel inhibition, BM adipocytes subsequently exhibited the increased expression of ATGL and HSL, which resulted in increased FFAs in supernatant (Figure 1F, G). Furthermore, it was found that RN1734 could significantly inhibit Ca\(^{2+}\) influx in BM adipocytes, while \(4\alpha\)PDD can promote Ca\(^{2+}\) influx in BM adipocytes (Supplementary Figure S1C). However, \(4\alpha\)PDD activates calcium channels in BM adipocytes by promoting Ca\(^{2+}\) influx, while the expression of TRPV4 could not increase (Supplementary Figure S2A).

To further confirm that TRPV4 regulates lipolysis of BM adipocytes, we used shTRPV4 lentivirus to knock down TRPV4 (Supplementary Figure S2B, C). As shown in Figure 1H, I and Supplementary Figure S2D, quantitative analysis showed that the number (Ctr vs shTRPV4, 528.1±46.4/mm\(^2\) vs 298.9±48.3/mm\(^2\), \(P < 0.05\)) and area
(Ctr vs shTRPV4, 798.7±57.5 µm² vs 454.7±54.0 µm², P < 0.01) of BM adipocytes decreased in TRPV4 knockdown samples. ATGL and HSL mRNA levels were also increased in TRPV4 knockdown adipocytes (Figure 1J). These data indicate a critical role for TRPV4 in the regulation of lipolysis in BM adipocytes.

**TRPV4 mediates GDF15-induced bone marrow adipocyte remodeling**

Increased lipolysis can result in a decrease in the number and area of BM adipocytes. Therefore, lipolysis is also a form of adipocyte remodeling. Our previous studies have found that GDF15 secreted by leukemia cells promoted BM adipocyte lipolysis, decreasing the number and area of BM adipocytes.\(^5\)\(^,\)\(^12\) As shown by Western blot analysis, TRPV4 expression was inhibited in BM adipocytes when co-cultured with leukemia cell lines (THP-1, K562, HL-60), whereas anti-GDF15 neutralizing antibodies partly reversed the effect (Figure 2A). Furthermore, based on the above results, we added recombinant human GDF15 (rhGDF15) into BM adipocytes to clarify this effect. The results showed that the inhibitory effect on TRPV4 was enhanced with the increase of rhGDF15 concentration and treatment duration (Figure 2B, C). Moreover, rhGDF15 could significantly inhibit TRPV4 mRNA expression and increase pHSL protein expression on the fourth day (Figure 2C and Supplementary Figure S2E). However, the pHSL protein and the release of FFAs did not increase significantly after BM adipocytes treated with rhGDF15 for 1 hour (Supplementary Figure S2F, G), suggesting that the role of GDF15 in promoting lipolysis may be different from the rapid action of β-adrenaline.\(^25\) ORO staining and quantitative analysis showed that the number and area of adipocytes did not change significantly on
the fourth day, but decreased significantly from the sixth day (Supplementary Figure S2H, I). These results indicate that TRPV4 regulates the remodeling of BM adipocytes.

To further explore the potential role of TRPV4 in GDF15-induced BM adipocyte remodeling, ORO staining and quantitative analysis showed that the number and area of BM adipocytes were decreased in BM adipocytes treated with rhGDF15, whereas 4αPDD partly reversed the effect of rhGDF15 (Figure 2D, E). OD value measurements of lipid droplets showed similar results (Figure 2F). Accordingly, rhGDF15 could induce increased expression of lipolysis genes (ATGL and HSL) and increased FFAs release in BM adipocytes, but activation of TRPV4 by 4αPDD partly reversed the effect of rhGDF15 (Figure 2G, H). Furthermore, rhGDF15 can inhibit Ca$^{2+}$ influx in BM adipocytes (Supplementary Figure S3A). These findings strongly suggest that TRPV4 contributes to GDF15-induced remodeling of BM adipocytes. Although GDF15 has been reported to act on Cav1.3, Cav3.1, Cav3.2, Cav3.3,13,26 their expression in BM adipocytes is much lower than TRPV4 (Supplementary Figure S3B). Moreover, when BM adipocytes were co-cultured with leukemia cell lines (THP-1, K562, HL-60), the expression of TRPV4 has significant changes (Supplementary Figure S3C). These results further suggest that TRPV4 may play an important role in GDF15-induced remodeling of BM adipocytes.

**GDF15 activates the downstream genes PI3K and pAKT in bone marrow adipocyte remodeling**

Extracellular GDF15 must bind to a receptor on the membrane surface to cause intracellular changes in BM adipocytes. We screened all of the reported GDF15
receptors by RT-qPCR and found that BM adipocytes mainly express \( TGF\beta RI \) and \( TGF\beta RII \) (Figure 3A). In order to determine whether GDF15 acts through binding to TGF\( \beta RI \) or TGF\( \beta RII \) on BM adipocytes, we conducted inhibitor experiments \textit{in vitro}.\textsuperscript{27,28} Western blot results showed that rhGDF15 could downregulate TRPV4 expression in BM adipocytes treated with Repsox (TGF\( \beta RI \) inhibitor) rather than ITD1 (TGF\( \beta RII \) inhibitor) (Figure 3B). Moreover, we found that rhGDF15 could reduce the number and area of BM adipocytes treated with Repsox as compared to that treated with ITD1 (Figure 3C, D). In accordance with data from RT-qPCR experiments, lipolysis genes (\( ATGL \) and \( HSL \)) were significantly elevated in BM adipocytes treated with RepSox compared with the level in BM adipocytes treated with ITD1 (Supplementary Figure S3D). These results suggest that TGF\( \beta RII \) is the major receptor that mediates GDF15 action on BM adipocytes.

To further verify the function of TGF\( \beta RII \) on BM adipocytes, we knocked down TGF\( \beta RII \) expression by shTGF\( \beta RII \) lentivirus (Supplementary Figure S4A, B). As shown in Figure 3E and F, when TGF\( \beta RII \) was knocked down in BM adipocytes, rhGDF15 did not significantly reduce the number and area of BM adipocytes. Accordingly, rhGDF15 did not significantly reduce the TRPV4 protein (Figure 3G). These results further confirmed that GDF15 regulates BM adipocyte remodeling by binding to TGF\( \beta RII \).

As an important signaling downstream pathway of the TGF\( \beta \) family, GDF15 could not cause significant changes in Smad2 and Smad4 proteins in BM adipocytes (Figure 3H and Supplementary Figure S4C). Notably, When PI3K was blocked by PI3K-IN-1
(PI3K inhibitor), rhGDF15 could not regulate AKT phosphorylation (Figure 3I), suggesting that GDF15 is involved in the remodeling of BM adipocytes by activating the PI3K/AKT pathway.

**The PI3K/AKT pathway inhibits the TRPV4 promoter FOXC1**

PI3K/AKT acts as a signaling pathway downstream of GDF15, which may affect the transcription or translation of TRPV4. We compared the expression of different transcription factors with or without rhGDF15 treatment by RNA-seq analysis. The results showed that the expression of several transcription factors decreased, including Forkhead box C1 (FOXC1), Spalt-like gene-2(SALL2), and MYC associated factor X (MAX) (Figure 4A). Based on the criteria of a fold change > 2.0 and P-value < 0.05, FOXC1 was identified as a transcription factor with significant changes in BM adipocytes after rhGDF15 treatment (Figure 4B). To identify that FOXC1 is responsible for GDF15 regulating TRPV4, we knocked down FOXC1 in BM adipocytes (Supplementary Figure S4D, E). The results showed that the expression of TRPV4 at both the mRNA and protein levels was inhibited in FOXC1 knockdown adipocytes (Figure 4C, D), suggesting that GDF15 reduced the expression of TRPV4 by negatively regulating the transcription factor FOXC1.

To further demonstrate the link between FOXC1 and TRPV4, we used a FOXC1 antibody to pull DNA fragments containing FOXC1 and used RT-qPCR to detect the TRPV4 gene in the fragment. The results showed that the control group had the sequence of the TRPV4 gene and the amount of TRPV4 gene was correspondingly decreased after knocking down FOXC1 (Figure 4E). These data suggest that FOXC1
directly combines with TRPV4. As shown in Figure 4F, rhGDF15 downregulated the expression of FOXC1 and TRPV4 protein, but PI3K-IN-1 can block this process. Taken together, these results again demonstrate that GDF15 regulates TRPV4 channels through the PI3K/AKT pathway.

**TRPV4 plays an important role in bone marrow adipocyte remodeling in acute myeloid leukemia mice**

To better understand the role of TRPV4 in leukemia cell-induced BM adipocyte remodeling, we investigated the changes in number and size of BM adipocytes with 4αPDD in leukemia mice. FBL-3 is a mouse-derived AML cell line, which can spontaneously induce leukemia. Firstly, we confirmed that FBL-3 cells secrete GDF15 (Supplementary Figure S5A). *In vitro*, FBL-3 cells co-cultured with BM adipocytes inhibited the expression of TRPV4 protein (Supplementary Figure S5B). Supplementary Figure S5C and D showed that FBL-3 cells reduced the number and area of BM adipocytes, while 4αPDD could partly reverse this effect. Correspondingly, FBL-3 cells can significantly promote the expression of *ATGL* and *HSL* mRNA in BM adipocytes, but 4αPDD can partly prevent this process (Supplementary Figure S5E). Additionally, 4αPDD had no significant effect on the proliferation and apoptosis of FBL-3 cells by CCK8 assay (Supplementary Figure S5F) and flow cytometry analysis (Supplementary Figure S5G). Thus, these results suggest that this dose of 4αPDD affects adipocytes, rather than directly affecting FBL-3 cells in the co-culture system.

Considering there were few adipocytes in the BM of C57BL/6 mice, we raised the mice with a high fat diet to increase the number of adipocytes in the BM. BM
adipocytes in obese mice are round or elliptical in shape (Figure 5A). Immunohistochemical (IHC) staining of perilipin1 protein in the BM adipocytes showed that the BM adipocytes stained yellow (Figure 5A). According to the pathogenic characteristics of FBL-3 cells,29 BM samples were taken after tail vein injected FBL-3 cells for 21 days. The shape of BM adipocytes did not change significantly, but the number and area were decreased (Figure 5A). Further quantitative analysis showed that the number of BM adipocytes in AML mice (219±37.7/mm²) was lower than that in the controls (505±49.7/mm²) and AML mice treated with 4αPDD (334.4±39.6/mm²), but the number of BM adipocytes in AML mice treated with 4αPDD was still lower than that in the controls (the t-test of any two groups showed that P<0.05) (Figure 5B). Similar results showed the BM adipocyte area in mice with leukemia was 860.0±142.5 µm², which was smaller than that in the controls (1686.4±106.7 µm², P<0.001). Meanwhile, the area of BM adipocytes in AML mice treated with 4αPDD was 1111.8±201.5 µm², which was larger than that of the BM adipocytes in AML mice (P<0.01), and did not return to normal (P<0.001) (Figure 5C).

We further found that CD117 (progenitor cell expression marker, red fluorescence) positive cells in AML mice were more than those in the AML mice treated with 4αPDD (Figure 5D). This may be due to the fact that GDF15 secreted by AML cells promotes lipolysis and is beneficial to the proliferation of leukemia cells, while 4αPDD partly prevents lipolysis. In fact, the content of GDF15 in the BM supernatant of AML mice was higher than that of the controls (Figure 5E), suggesting that GDF15 secreted by leukemia cells can promote lipolysis of BM adipocytes. Furthermore, we observed that
treatment with 4αPDD significantly extended overall survival of AML mice (Figure 5F). In short, these results suggest that targeting TRPV4 in BM adipocytes can delay the progression of leukemia in mice.

**Discussion**

We have demonstrated a possible mechanism whereby TRPV4 mediates BM adipocytes responses to extracellular GDF15. Our data showed that AML cells drive this remodeling process, at least in part, through TRPV4-dependent lipolysis in the adipocytes. Our previous reports linked increased levels of small adipocytes in BM to poor prognosis in AML patients, and revealed that GDF15 derived from leukemia cells remolds mature BM adipocytes into small adipocytes.5,12 Here, we found that, GDF15 binds to its receptor TGFβRII on BM adipocytes, which in turn activates downstream target genes, including PI3K and AKT. Subsequently, TRPV4 is inhibited via downregulation of its transcription factor FOXC1. These results suggest that GDF15 regulates TRPV4 through the above pathway, thereby promoting BM adipocyte remodeling (Supplementary Figure S6). The finding is consistent with several reports that TRPV4 acts as a volume receptor rather than an osmotic receptor.30-32

There has been a report that TRPV4 is located on the cell membrane and acts as a calcium channel.33 Therefore, TRPV4 can regulate energy metabolism of peripheral white adipocytes by facilitating Ca^{2+} influx, which in turn stimulates the ERK1/2-dependent pathway.34 GDF15 inhibits the expression of TRPV4 in BM adipocytes, resulting in a decrease of Ca^{2+} influx (Supplementary Figure S3A) and an increased in pHSL protein (Supplementary Figure S2E) after BM adipocytes treated
with rhGDF15 for four days. It has been reported that reduced Ca\(^{2+}\) influx causes an increase in the expression of pHSL, leading to lipolysis of adipocytes.\(^{17}\) Notably, the TRPV4 channel is a tetrameric complex formed by the same or similar monomeric subunits.\(^{35}\) Interestingly, cytosolic N- and C-terminal domains are involved in channel gating and mediating intracellular signaling,\(^{35,36}\) indicates that it is impossible for TRPV4 to directly interact with exogenous chemical factors. Hence, it would be interesting to examine how TRPV4 communicates with extracellular GDF15.

Given our findings that extracellular GDF15 inhibited the expression of TRPV4 in BM adipocytes, we speculated that GDF15 acts on BM adipocytes through TGF\(\beta\) receptors. As a member of the TGF\(\beta\) superfamily, GDF15 is known to interact with receptors of the TGF\(\beta\) members, such as TGF\(\beta\)RI, TGF\(\beta\)RII, ALK4 and ACVR2.\(^{37-39}\) In addition, GDF15 has unique cognate receptors, such as GFRAL, which is mainly expressed in the central nervous system and, at low levels, in the testicular tissue.\(^{39}\) Our data showed that BM adipocytes express TGF\(\beta\) receptors, but not the known unique GDF15 receptors. Moreover, TGF\(\beta\)RII was shown to be associated with GDF15 activity on BM adipocytes (Figure 3B-G). These experiments inform the first step of GDF15 acting on the adipocytes.

Our study further revealed that PI3K/AKT activation plays an essential role in driving GDF15 regulation of target genes in BM adipocytes. In fact, GDF15 induced the activation of Smad, a component of the classic anti-apoptosis pathway of cardiomyocytes which promotes the progression of lung cancer.\(^{40,41}\) However, we did not focus on the Smad pathway in this study because the activated Smad protein type is
known to be determined by the TGFβRI present in the ligand-bound signal complex. In fact, GDF15 did not affect the Smad signaling pathway in BM adipocytes, which is consistent with our results (Figure 3H and Supplementary Figure S4C). Taken together, our data, when interpreted in the context of previous reports, suggest that the PI3K/AKT pathway may be important for GDF15 induced remodeling of BM adipocytes.

Interestingly, we observed that PI3K/AKT activation downregulated the TRPV4-associated transcription factor FOXC1. FOXC1 is also a transcription factor of ITGA7 and FGFR4 in colorectal cancer, CXCR4 in endothelial cells, and FGF19 in ciliary body-derived cells. Moreover, the transcriptional function of FOXC1 has not been described previously for some pivotal adipogenic genes (FABP4, CEBPα and PPARγ) and lipolytic genes (ATGL and HSL). Based on the knockdown of FOXC1 gene, the lipolytic gene in BM adipocytes increases (Supplementary Figure S4F), which confirmed that FOXC1 is important for regulating the metabolism of BM.

In addition, TRPV4 can be activated or inhibited by physical and chemical factors. when it comes to the matter of size of cells, TRPV4 acts as a volume receptor rather than an osmotic receptor, suggesting that TRPV4 is involved in the regulation of cell volume. Previous studies have suggested that TRPV4 is an important inflammatory factor because TRPV4 levels are increased in inflammatory tissues and activation of TRPV4 causes inflammation. This protein is also closely related to inflammation of white adipose tissue. But the decreased expression of TRPV4 in leukemia-associated BM adipocytes implicates that TRPV4 is not a major
pro-inflammatory factor of leukemia-associated BM adipocytes. We conclude that downregulated TRPV4 preferentially promotes lipolysis in BM adipocytes, contributing to their remodeling into small adipocytes in AML pathogenesis.

Moreover, we also found that treatment with the TRPV4 agonist 4αPDD rescued BM adipocyte remodeling, which was correlated with increased survival in AML bearing mice, supporting a crucial role of TRPV4 in growth and progression of AML. Although there are unstained positive white circles in the BM of AML mice (Figure 5A), we suspect that these circles may represent an increase in blood vessels or sinuses in the BM of AML mice. Because leukemia cells can promote angiogenesis, which in turn contribute to the proliferation of leukemia cells. Of course, it could not be excluded that few adipocytes were not stained positively. Many studies have also reported the effect of circulating factors in obese animals on leukemia. However, in our study, both experimental groups and control groups were obese mice, and their basic background was the same. Thus, it should allow us to control the impact of the circulating factors and have no effect on our observation of mouse survival and other variables. Future studies in vivo are needed to validate the specific modulatory role of GDF15 on TRPV4.

In conclusion, leukemia cells activate a transcriptional network that includes GDF15 related-PI3K/AKT activation and subsequent TRPV4 downregulation that promotes BM adipocyte remodeling. The morphological adaptation of BM adipocytes and the modulation of lipolysis may represent a novel strategy for the treatment of hematological malignancies, especially in elderly patients, whose aging and increased
adiposity of the BM microenvironment reduces the efficacy of cytotoxic chemotherapy.

**Conflict of interest**

The authors declare no conflict of interest.

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Abbreviations: TRPV, transient receptor potential vanilloid; ALK4, activin A receptor type 1B; ACVR2, activin receptor type 2; TGFβRII, transforming growth factor-β type II receptor; TGFβRI, transforming growth factor-β type I receptor; GFRAL, GDNF family receptor α-like; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive triglyceride lipase; FOXC1, Forkhead box C1; Cav1.3, calcium voltage-gated channel subunit alpha1 C; Cav3.1, calcium voltage-gated channel subunit alpha1 G; Cav3.2, calcium voltage-gated channel subunit alpha1 H; Cav3.1, calcium voltage-gated channel subunit alpha1 I.
Figure legends

Figure 1. Downregulated TRPV4 contributes to increased bone marrow adipocyte lipolysis. (A) RT-qPCR verification of the expression of transient receptor potential vanilloid (TRPV) channel genes in bone marrow (BM) adipocytes. (B) Western blot analysis of TRPV4 protein in BM adipocytes from three patients. (C) BM adipocytes treated with dimethyl sulfoxide (Ctr), RN1734 (5 μM) or 4α-phorbol 12,13-didecanoate (4αPDD, 0.25 μg/mL) for 4 days. Adipocytes were stained by Oil red O (ORO) staining. All images were at a magnification of 200×. (D) The number and average area of BM adipocytes from the indicated groups were measured by using Image-Pro-Plus 5.1. (E) The content of lipid-droplets in BM adipocytes from the indicated groups was detected by optical density (OD) values after ORO staining. (F) RT-qPCR was used to analyze adipose triglyceride lipase (ATGL) and hormone-sensitive triglyceride lipase (HSL) mRNA in BM adipocytes from the indicated groups. (G) The content of free fatty acids (FFAs) in the supernatant of BM adipocytes treated with dimethyl sulfoxide (Ctr), RN1734 or 4αPDD was detected using the colorimetric method. (H) BM adipocytes were infected with TRPV4-targeted shRNA (shTRPV4) lentivirus for 6 days. Adipocytes were stained with Alexa Fluor 493/503-conjugated BODIPY. DAPI was stained blue and lipid droplet was green fluorescent. Scale bar represents 50 μm. (I) Quantitative analysis of the number and area in BM adipocytes infected with shTRPV4 lentivirus by Image-Pro-Plus 5.1. (J) The mRNA level of HSL and ATGL in BM adipocytes infected with shTRPV4 lentivirus on the fourth day was detected by RT-qPCR. β-actin protein was used as an
internal control for Western blot analysis. Three independent experiments were performed. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Figure 2. TRPV4 mediates GDF15-induced bone marrow adipocyte remodeling. (A) BM adipocytes were co-cultured with leukemia cell lines (THP-1, K562, HL-60) or leukemia cells and anti-GDF15 neutralizing antibody (200 ng/mL) for 4 days. The protein of TRPV4 was detected using Western blot analysis. (B) The effect of different concentrations (100 ng, 200 ng, 500 ng) of recombinant human GDF15 (rhGDF15) on the expression of TRPV4 protein for 4 days was analyzed by Western blot. (C) RT-qPCR was performed to analyze the expression of TRPV4 mRNA after the addition of 200 ng rhGDF15 in BM adipocytes for 2, 4, 6, 8 days. (D) BM adipocytes treated with dimethyl sulfoxide (Ctr), rhGDF15 (200 ng/mL) or rhGDF15 (200 ng/mL) and 4αPDD (0.25 μg/mL) for 6 days. Adipocytes were stained by ORO staining. All images were at a magnification of 200×. (E) The number and average area of adipocytes from the indicated groups were measured by using Image-Pro-Plus 5.1. (F) The content of lipid droplets in the indicated groups was detected by OD values. (G) RT-qPCR was used to analyze HSL and ATGL mRNA in adipocytes from the indicated groups on the fourth day. (H) The content of FFAs in the supernatant of BM adipocytes from each group was detected using a colorimetric method. β-actin protein was used as an internal control for Western blot analysis. Three independent experiments were performed. **$P < 0.01$, *$P < 0.05$.

Figure 3. GDF15 activates the downstream target gene PI3K and pAKT in bone marrow adipocyte remodeling. (A) RT-qPCR analysis of GDF15-related receptors in
BM adipocytes. (B) Western blot analysis of the expression of TRPV4 protein in BM adipocytes induced by rhGDF15 after treatment with TGFβRI inhibitor (RepSox) or TGFβRII inhibitor (ITD1) for 4 days. (C) ORO staining analysis of BM adipocytes induced by rhGDF15 after treatment with RepSox and ITD1 for 6 days. All images were at a magnification of 200×. (D) The number and average area of BM adipocytes from the indicated groups were measured by using Image-Pro-Plus 5.1. (E, F) BM adipocytes were infected with TGFβRII-targeted shRNA (shTGFβRII) lentivirus for 48 hours (h) and then cultured with rhGDF15 for 6 days. Adipocytes were stained with Alexa Fluor 493/503-conjugated BODIPY. DAPI was stained blue and lipid droplet was green fluorescent. The number and average area of adipocytes from the indicated groups were measured by using Image-Pro-Plus 5.1. Scale bar represents 50 μm. (G, H) BM adipocytes were infected with shTGFβRII lentivirus for 48 h and then cultured with rhGDF15 for 4 days. The protein level of TRPV4, Smad2 and pSmad2 was detected using Western blot analysis. (I) BM adipocytes were treated with or without rhGDF15 and PI3K inhibitor (PI3K-IN-1, 2 μM) for 4 days. The protein level of PI3K, AKT and pAKT was detected using Western blot analysis. β-actin protein was used as an internal control for Western blot analysis. Three independent experiments were performed. **P < 0.01, *P < 0.05.

Figure 4. The PI3K/AKT pathway inhibits the TRPV4 promoter FOXC1. (A) Analysis of TRPV4 upstream transcription factor expression of BM adipocytes treated with or without rhGDF15 for 2 days by RNA sequencing (RNA-Seq). (B) Different expression of TRPV4-related transcription factor genes following rhGDF15 treatment.
for 2 days. (C) RT-qPCR was used to analyze TRPV4 mRNA level after treatment with FOXC1-targeted shRNA (shFOXC1) lentivirus for 48 h. (D) Western blot was used to analyze the expression of TRPV4 protein after treatment with shFOXC1 lentivirus for 48 h. (E) Chromatin immunoprecipitation-qPCR (Chip-qPCR) analysis of TRPV4 gene level in adipocytes with or without FOXC1 knockdown. (F) BM adipocytes were treated with or without rhGDF15 and PI3K inhibitor (PI3K-IN-1, 2 μM) for 4 days. The protein level of FOXC1 and TRPV4 was detected using Western blot analysis. β-actin protein was used as an internal control for Western blot analysis. Three independent experiments were performed. **P < 0.01, *P < 0.05.

Figure 5. TRPV4 plays an important role in bone adipocyte remodeling in acute myeloid leukemia mice. (A) H&E staining and Immunohistochemical (IHC) staining with perilipin1 antibody in BM sections from the controls (Ctr, n=5) and experimental mice (AML mice, n=5; AML mice treated with 4αPDD, n=5). Ten fields were analyzed for each mouse at 400× magnification. Three independent experiments were performed. Scale bars represent 50 μm and 100μm, respectively. (B, C) Quantitative analysis of the adipocyte number and area in the controls, AML mice and AML mice treated with 4αPDD by Image-Pro-Plus 5.1. (D) Immunofluorescence was used to analyze the expression of CD117 in BM sections of the controls, AML mice and AML mice treated with 4αPDD. DAPI was stained blue and CD117 was red fluorescent. Scale bars represent 100 μm. (E) The GDF15 content of the BM supernatant in the controls and AML mice were analyzed by ELISA. (F) Kaplan-Meier curves showing the overall survival rate of AML mice (n=9) and AML mice treated with 4αPDD (n=9). Three
independent experiments were performed. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. 
Supplementary method

Cell culture and reagents

The leukemia cell lines THP-1, K562 and HL-60 were purchased from the Chinese Academy of Sciences Cell Bank, Shanghai, China. THP-1, K562 and FBL-3 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, contains L-Glutamine). HL-60 cells were cultured in Iscove's modified Dulbecco's medium (IMDM, Gibco, contains 3g/L D-Glucose, L-Glutamine, HEPES). Mesenchymal stem cells (MSCs) were cultured in low glucose Dulbecco’s modified Eagle’s medium (DMEM, Gibco, contains 1g/L D-Glucose, L-Glutamine, 110mg/L Sodium Pyruvate). Adipocytes were cultured in high glucose DMEM (Gibco, contains 4.5g/L D-Glucose, L-Glutamine, 110mg/L Sodium Pyruvate). All of the above media contained 10% fetal bovine serum (FBS, Gibco), 1% L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. All these cells were cultured at 37°C in 5% CO₂. RN1734 (MCE, HY19975, USA) and 4αPDD (MCE, HY18739, USA) are inhibitors and agonists of TRPV4, respectively. BM adipocytes were treated with rhGDF15 (Peprotech 120–28, USA), a neutralizing anti-GDF15 antibody (R&D Systems, MAB957, USA), Repsox (MCE, HY13012,USA), ITD1 (MCE, HY12704,USA) or PI3K-IN-1(MCE, HY12068, USA) to observe the effect of GDF15.

Adipogenic induction of Mesenchymal stem cells

All BM adipocytes used in vitro were derived from the induction into adipogenesis by MSCs. MSCs were planted with 5×10⁵ into 24-well plates. To induce adipogenic differentiation, MSCs were treated with an adipogenic medium A consisting of high
glucose DMEM supplemented with 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine (Sigma-Aldrich, USA), 0.2 mM indomethacin (Sigma-Aldrich, USA), 1 mM dexamethasone (Sigma-Aldrich, USA) and 10 mM insulin (Sigma-Aldrich, USA) for three days. Subsequently, MSCs were treated with an adipogenic medium B consisting of high glucose DMEM supplemented with 10% FBS and 10 mM insulin (Sigma-Aldrich, USA) for one day. This is one cycle of the induction. Finally, three cycles were conducted and maintained the induced adipocytes in high glucose DMEM with 10% FBS.

**Co-culture assay**

Differentiated BM adipocytes were placed in a normal growth medium until the co-culture experiment. For co-culture experiments, leukemia cell lines (THP-1, HL-60, K562, FBL-3) were harvested, resuspended in serum medium and planted on adipocytes of the Transwell system (0.4 µm, Corning, USA) at a density of 5×10^5 per well in 1 mL RPMI 1640 medium or IMDM medium containing 10% FBS. The adipocyte/leukemia cell co-cultures were then incubated for 4 days.

**Free fatty acids detection**

The adipocytes were cultured in DMEM high glucose medium containing 10% FBS. Dimethyl sulfoxide (DMSO), RN1734 (5 µM), 4αPDD (0.25 µg/mL) and rhGDF15 (200 ng/mL) were added to the above medium according to the experimental design. The supernatant of BM adipocytes was collected. The free fatty acids (FFAs) in the supernatant were determined using a free fatty acid kit (FUJIFILM Wako Pure Chemical Corporation, Japan).
**Lentiviral knockdown**

Scrambled lentivirus was used as the lentivirus control (Control shRNA lentivirus, Ctr). TRPV4-targeted shRNA (shTRPV4), TGFβRII-targeted shRNA (shTGFβRII) or FOXC1-targeted shRNA (shFOXC1) lentivirus were purchased from Jikai Biotechnology Co., Ltd. (Shanghai, China). MSCs were plated at a density of $5 \times 10^5$ /well in 24-well plates and were induced into adipocytes. After 24 h, BM adipocytes were infected with shTRPV4, shTGFβRII and shFOXC1 lentiviral stock. RT-qPCR and Western blot were used to analyze BM adipocytes infected for 48 h.

**Calcium measurement**

Fluo 3-AM (excitation wavelength, 506 nm; emission wavelength, 526 nm, AAT Bioquest, USA) was used to measure the intracellular calcium levels. BM adipocytes were cultured with rhGDF15 (200 ng/mL) for 4 days and 8 days. BM adipocytes were treated with RN1734 (5 µM) and 4αPDD (0.25 µg/mL) for 4 days. Calcium levels with treatment of 1 µg/mL Fluo 3-AM were determined by flow cytometry analysis of aliquots of BM adipocytes. The cells were incubated at 37°C for 30 min with shaking, washed with Hank's Balanced Salt Solution (HBSS). Adipocytes that were not incubated with Fluo 3-AM were used as a negative control group. Ca$^{2+}$-dependent Fluo3-AM fluorescence intensity was measured by flow cytometry (BD, USA).

**RNA sequencing**

BM adipocytes that had been treated with or without recombinant human GDF15 (rhGDF15) for two days were used for this study, according to the manufacturer’s instructions. Briefly, total RNA was isolated using a RNeasy micro kit (Shanghai
Qiming Information Technology Company Limited, China), and used for double-stranded cDNA synthesis and library amplification. RNA sequencing (RNA-seq) libraries were run on an Illumina HiSeq Xten next-generation sequencer using paired-end 150bp sequencing. Red and green signals indicate high and low expression of each gene, respectively.

**Western blot analysis and ELISA**

Total cellular proteins were extracted with mixed RIPA lysate. Anti-TRPV4 (1:1000, Sigma SAB2104216, USA), anti-PI3K (1:1000, Abcam 40755, USA), anti-pAKT (1:1000, CST4060T, USA), anti-AKT (1:1000, Abcam 179463, USA), anti-Smad2 (1:1000, CST5339, USA), anti-pSmad2 (1:1000, CST18338, USA), anti-Smad4 (1:1000, Abcam 40759, USA), anti-pSmad4 (1:500, Invitrogen PA5-64712, USA), anti-FOXC1 (1:1000, Abcam 5079, USA), anti-HSL (1:1000, Abcam 45422, USA), anti-pHSL (1:1000, Abcam 109400, USA) and anti-β-actin (1:1000, A20120A0702, BioTNT, China) were used. Secondary antibodies were used at 1:10000 (goat anti-rabbit IgG H+L HRP, A20120A0704, BioTNT, China; goat anti-mouse IgG H+L HRP, A20120A0703, BioTNT, China). The FBL-3 cell supernatant was obtained from cells cultured in RPMI 1640 medium with 10% FBS at different cell densities (2×10^5/mL, 5×10^5/mL). The BM fluid of normal obese mice and AML mice was washed with PBS (1 mL). The ELISA analysis for mouse GDF15 was performed according to the manufacturer’s instructions (R&D, MGD150, USA).

**Quantitative reverse transcription PCR**
Total RNA was extracted using TRIzol reagent (Invitrogen, UK), and RNA samples were converted into cDNA using the Primer Script TM RT reagent Kit (Perfect Real Time, TaKaRa, Japan). All real-time PCR reactions were performed using an ABI 7500 real-time PCR system (Biosystems, Foster City, CA, USA) and the SYBR Premix Ex Taq reagent kit (RR420A, TaKaRa, Japan). The primer sequences are shown in Table1. The results were analyzed using the 2^{ΔΔCT} method and GAPDH to normalize the results.

**Cell Counting Kit-8 assay**

FBL-3 cells (3×10^3) were plated into 96-well culture plates. Different concentrations of 4αPDD (0 µg/mL, 0.25 µg/mL) were added. The FBL-3 cell proliferation was tested by a Cell Counting Kit-8 assay (CCK8) kit (Dojindo, Japan). 10 µL CCK8 reagent was added to each well and incubated for 2 h at 37 °C. The measurement of absorption at 450 nm was performed using a microplate reader.

**Apoptosis assay**

FBL-3 cells were treated with 4αPDD (0 µg/mL, 0.25 µg/mL) for 48 h. FBL-3 cells were sorted and double labelled with Annexin V-APC and propidium iodide (PI) (Invitrogen by Thermo Fisher Scientific, USA) to analyze apoptosis, according to the manufacturer’s instructions.

**Oil red O staining**

MSCs were planted with 5×10^5 into 24-well culture plates, followed by induction of adipogenesis. BM adipocytes were treated with dimethyl sulfoxide, RN1734 (5 µM), 4α-phorbol 12,13-didecanoate (4αPDD, 0.25 µg/mL), rhGDF15 (200 ng/mL), Repsox (25 µM) and ITD1 (1 µM). BM adipocytes were fixed in 4% formaldehyde solution for
30 min, washed with PBS for 5 min and stained with Oil red O (ORO) solution (Solarbio, China) for 30 min. We captured the images under an ordinary optical microscope (Olympus, Japan). All images were magnified for 200 times. Subsequently, isopropyl alcohol (500 μL) was added to each well and the eluted ORO was quantified through measurement of the absorption at 490 nm using a microplate reader (Thermo Fisher, USA).

**Immunofluorescence**

BM adipocytes were treated with shTRPV4 and shTGFβRII for 6 days, and fixed with 4% paraformaldehyde. Alexa Fluor 493/503-conjugated Bodipy (Sigma, USA) was added at a concentration of 2 μg/mL, and stained for 30 min at room temperature. BM adipocytes treated with FBL-3 cells and 4αPDD (0.25 μg/mL) were also stained as described above. BM sections of normal obese mice, AML mice and AML mice treated with 4αPDD were stained with CD117 (rabbit anti-CD117 monoclonal antibody, SAB, USA). DAPI was then dyed for 15 min at room temperature. Fluorescent images were captured using a fluorescence microscope system (Olympus, Japan).

**Adipocyte measurement**

The number and area of adipocytes were analyzed by Image-Pro-Plus 5.1 software. The resolution of the analysis software is 2048×1536 pixels (408×308 μm). 33×25 meshes could be automatically generated on the images. Each crossing between vertical and horizontal lines was recorded as a point. The software automatically stored the number of positive adipocytes and calculated the corresponding results.
Supplementary Figure S1. TRPV4 regulates intracellular calcium. (A) Dose response for the cellular viability of bone marrow (BM) adipocytes treated with RN1734. The IC50 values were calculated using a Nonlinear regression analysis. (B)
Histogram showed the Fluo3-AM fluorescence in BM adipocytes as a measure of the cytosolic free Ca\(^{2+}\) concentration. BM adipocytes treated with different concentrations of 4α-phorbol 12,13-didecanoate (4αPDD). The Fluo 3-AM level of calcium was measured by flow cytometry. (C) Histogram showed the Fluo3-AM fluorescence in BM adipocytes as a measure of the cytosolic free Ca\(^{2+}\) concentration. BM adipocytes treated with RN1734 and 4αPDD for 10 seconds (s) and 4 days. The Fluo 3-AM level of calcium was measured by flow cytometry. Three independent experiments were performed.
Supplementary Figure S2. GDF15 promotes remodeling of bone marrow adipocytes. (A) Western blot was used to verify the expression of TRPV4 in BM adipocytes treated with 4αPDD. (B) RT-qPCR analysis of the expression of TRPV4 mRNA in BM adipocytes infected with shTRPV4 lentivirus for 48 hours (h). (C)
Western blot analysis of the knockdown efficiency of TRPV4 protein in BM adipocytes.

(D) BM adipocytes were infected with shTRPV4 lentivirus for 6 days. Adipocytes were stained by Oil red O (ORO) staining. (E, F) showed the expression of HSL and pHSL in BM adipocytes treated with recombinant human GDF15 (rhGDF15) for 4 days and 1 h by Western blot analysis. (G) The content of FFAs in the supernatant of BM adipocytes treated with rhGDF15 for 1 h was detected using the colorimetric method. (H) BM adipocytes treated with rhGDF15 (200 ng/mL) for 0, 4, 6, 8 days. Adipocytes were stained by Oil red O (ORO) staining. All images were at a magnification of 200×. (I) The number and average area of adipocytes from the indicated groups were measured by using Image-Pro-Plus 5.1. Three independent experiments were performed. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. 
Supplementary Figure S3. The effect of GDF15 on calcium channels in bone marrow adipocytes. (A) Histogram showing the Fluo3-AM fluorescence in BM adipocytes as a measure of the cytosolic free Ca$^{2+}$ concentration. BM adipocytes treated with rhGDF15 for different time (4 days and 8 days). The Fluo 3-AM level of calcium
was measured by flow cytometry. (B) RT-qPCR was used to analyze the relative expression of Cav1.3, Cav3.1, Cav3.2, Cav3.3 and TRPV4 in BM adipocytes. (C) RT-qPCR were used to analyze the expression of Cav1.3, Cav3.1, Cav3.2, Cav3.3 and TRPV4 in BM adipocytes co-cultured with leukemia cell lines (THP-1, K562, HL-60). (D) RT-qPCR was used to analyze HSL and ATGL mRNA in BM adipocytes induced by rhGDF15 after treatment with RepSox or ITD1 for 4 days. Three independent experiments were performed. **P < 0.01, *P < 0.05.
Supplementary Figure S4. Validation of knockout efficiency of TGFβRII and FOXC1 genes. (A) RT-qPCR analysis of the expression of TGFβRII mRNA in BM adipocytes infected with shTGFβRII lentivirus for 48 h. (B) Western blot analysis of the knockdown efficiency of TGFβRII protein in BM adipocytes. (C) BM adipocytes were infected with shTGFβRII lentivirus for 48 h and then cultured with rhGDF15 for
4 days. The protein of Smad4 and pSmad4 was detected using Western blot analysis. THP-1 cells were used as positive control. (D) RT-qPCR analysis of the expression of FOXC1 in BM adipocytes infected with shFOXC1 lentivirus for 48 h. (E) Western blot analysis of the efficiency of FOXC1 knockdown in BM adipocytes infected with shFOXC1 lentivirus. (F) RT-qPCR was used to analyze FABP4, PPARγ, CEBPa, ATGL and HSL mRNA levels after treatment with shFOXC1 lentivirus for 48 h. Three independent experiments were performed. **P < 0.01, *P < 0.05.
Supplementary Figure S5. FBL-3 cells remodel bone marrow adipocytes in vitro.

(A) Concentration of GDF15 in supernatant of FBL-3 cells (2×10⁵/mL and 5×10⁵/mL) by ELISA at different time (1, 2, 3 days). (B) Western blot analysis of the expression of TRPV4 protein after co-cultured of FBL-3 cells with BM adipocytes for 4 days. (C, D)
BM adipocytes co-cultured with dimethyl sulfoxide (Ctr), FBL-3 (2×10⁵/mL) or FBL-3 (2×10⁵/mL) and 4αPDD (0.25μg/mL) for 6 days. Adipocytes were stained by ORO staining. All images were at a magnification of 200×. The number and average area of adipocytes from the indicated groups were measured by using Image-Pro-Plus 5.1. (E) BM adipocytes co-cultured with dimethyl sulfoxide (Ctr), FBL-3 (2×10⁵/mL) or FBL-3 (2×10⁵/mL) and 4αPDD (0.25μg/mL) for 4 days. RT-qPCR was used to analyze ATGL and HSL mRNA. (F) CCK8 assay was used to analyze the effect of 4αPDD (0μg/mL and 0.25μg/mL) on FBL-3 cell proliferation. (G) Flow cytometry analysis of the apoptosis of FBL-3 cells treated with 4αPDD (0.25μg/mL). Three independent experiments were performed. ***P < 0.001, **P < 0.01, *P < 0.05.
Supplementary Figure S6. Patterns of TRPV4 in bone marrow adipocytes regulated by GDF15 derived from leukemia cells. MSCs were derived from lymphoma patients without BM invasion and were induced into mature adipocytes in vitro. GDF15 secreted by leukemia cells binds to TGFβRII on BM adipocytes. Subsequently, the PI3K/AKT pathway in the adipocytes is activated, thereby inhibiting the expression of the transcription factor FOXC1. This series of processes leads to a decrease in the expression of TRPV4 in BM adipocytes and promotes BM adipocyte remodeling. Thus, Ca^{2+} influx ([Ca^{2+}]_{i}) is reduced, which promotes the expression of
ATGL and HSL in BM adipocytes. Eventually, BM adipocytes undergo lipolysis and remodeling.