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Experimental Study on Sdccag3 Regulating Osteogenesis and Adipogenic Differentiation of Rats Bone Marrow Mesenchymal Stem Cells

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Abstract

Background

Bone marrow mesenchymal stem cells have a metabolic balance between osteogenic and adipogenic differentiation. Sdccag3 is differentially expressed in hyperlipidemia rats, and it can be beneficial to the osteogenesis disorder caused by dyslipidemia, but the pathway mechanism and its influence on the differentiation have not been studied.

Methods

Here, we designed RT-PCR and Western Blot to determine the expression of Osteogenic and lipid gene, including ALP, Runx2, PPARγ FABP4 and so on, and then we performed microarray to evaluate the bone formation, calculate BV/TV. Simultaneously, we detected mRNA from the hyperlipidemia rat model we established to find the specific pathway mechanism.

Results

In this experiment, we found that high fat environment influences BMSCs differentiation. Sdccag3 overexpression upregulates the osteogenic differentiation of BMSCs and increased new bone formation.

Conclusion

Therefore, our findings show that Sdccag3 regulates osteogenesis and adipogenic differentiation of BMSCs.

Background:

Bone marrow mesenchymal stem cells (BMSCs) are common precursors of osteoblasts and adipose cells, as well as other cell types, when they are induced by hyperglycemia or stress signals, is more inclined to adipocyte lineage than osteoblast lineage, resulting in bone adipogenesis or the phenotype of osteoporosis. The balance between the two differentiation pathways in bone marrow is changed, which promotes the accumulation of fat in bone marrow at the expense of osteoblast formation [1].

Wnt signaling pathway plays an important role in stem cell biology, homeostasis and many diseases. Secreted Wnt proteins activate different downstream pathways that play a central role in bone development. Frizzled low Density Lipoprotein receptor-related protein 5 (LRP5) triggers transcriptional coactivator β-catenin to be transported to the nucleus and interacts with related transcription factors to regulate various target genes of Wnt [2]. Wnt family proteins 5a/ 5b (Wnt5a/Wnt5b) are key proteins in the

non-classical pathway of Wnt signaling. Studies have found that both Wnt5a and Wnt5b are expressed in chondrocytes of developing long bones in mice and regulate bone development in vivo [3].

Sdccag3 (serologically defined colon cancer antigen3) is an endosomal protein that locates in early and recoverable endosomes. Previous experiments showed that high lipid environment inhibited BMSCs differentiation into osteoblasts [4]. At the same time, the membrane curl receptor protein Fzd9 on the Wnt pathway was also inhibited in the high-fat environment [5]. In addition, Sdccag3 plays an important role in bone metabolism and regulates bone formation in dyslipidemia. This is the first time that Sdccag3 has been found to play a functional role in bone. However, the effects of Sdccag3 on osteoblast and lipid metabolism homeostasis of bone marrow mesenchymal stem cells have not been studied yet.

With the continuous improvement of people's living standards, hyperlipidemia has become one of the common pathological diseases in human beings [6]. Hyperlipidemia refers to abnormally elevated levels of lipids or lipoproteins in the blood due to abnormal fat metabolism or function, which is characterized by a decrease in circulating high-density lipoprotein cholesterol (HDL-C),and increase in Total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C)[7].A large number of epidemiological studies have shown a positive correlation between cardiovascular disease and the risk of osteoporosis [8–11], suggesting a link between hyperlipidemia and abnormal bone metabolism, and that patients with hyperlipidemia are at increased risk of osteoporosis. On the other hand, Currently, dental implants are increasingly widely used in the treatment of partial or total tooth loss. Despite the success of dental implants and high survival rate, the prevalence of diseases around dental implants has increased [12].

Therefore, the purpose of this study was to explore the effects of hyperlipidemia on osteogenic and lipid metabolism homeostasis of bone tissue and bone marrow mesenchymal stem cells around implant in rats, what's more, the changes of osteogenic and lipid metabolism homeostasis under the regulation of Sdccag3 and further studies on the pathways and mechanisms that Sdccag3 affects bone metabolism.

Materials And Methods:

Extraction of the BMSCs

The tibia and femurs were sterile dissected from 3-week-old Wistar rats, and bone marrow cells were harvested, cultured and passaged using 15% fetal bovine serum (FBS), DMEM medium. Cells passed to the third generation were used for subsequent experiments.

Osteogenic induction of bone marrow mesenchymal stem cells

The third generation BMSCs were counted and laid in 6-well plates at 3×10^5 cells per well. When the growth congregation reached 80%, the cells were randomly divided into the high-fat group and the normal group. Osteogenic induced medium consisting of DMEM medium, vitamin C, β-glycerol phosphate. Fresh

induction solution was replaced 2-3 days per time, and the corresponding experimental detection was carried out at the corresponding time.

Transfection and viral infection

After the 3rd generation BMSCs cells were counted, 3×10^5 cells/well were laid on the 6-well plate, and 3ml of complete medium was added. When the cells were fused to 40%~60%, the virus was added according to the optimal MOI value after the solution was changed, and the virus was gently blown and mixed, and incubated in the incubator. They are divided into four groups: Sdccag3-knockdown, negative control LV16 and Sdccag3-overexpression, negative control LV17 lentivirus vector.

The cells were examined 8-12 hours later, and the fluid was changed on the second day. After virus infection for 96h, the cells were replaced with ordinary/high fat osteogenic induction solution and induced to the corresponding time for the corresponding experimental detection.

Reverse transcription-quantitative PCR (RT-PCR)

Total RNA was extracted from BMSCS using Trizol reagent and reverse transcribed to cDNA by using PrimeScript RT Master Mix and transcript levels were quantified using SYBR Green PCR Master Mix. Gene expression levels were quantified using the 2- ΔΔCt method.

Protein blotting(Western Blot)

Cells were lysed in RIPA lysate, proteins separated by 10%SDS-page and transferred to PVDF and membrane blocked in skim milk (5%) at 1h at room temperature. Then incubated with the primary antibody at 4℃ through the night. TBST was washed three times for ten minutes each, and the corresponding secondary antibodies were incubated for 1h at room temperature. Protein levels were determined by the chemiluminescence method.

Immunofluorescence

The cells were immobilized with 4% paraformaldehyde for 20min, 0.2%Triton x-100 permeation and 1% goat serum for 30 min. Then the cells were incubated with primary antibody for 4 nights and fluorescent-coupled secondary antibody was added for 1 hour. Goat serum was closed for 30 minute and primary antibody was added for incubation. The cells were incubated with fluorescent-coupled secondary antibody at room temperature for 1 hour and incubated with DAPI for 5 minutes to stain the nucleus. Photographs are taken through a laser scanning microscope.

Alkaline phosphatase (ALP), Alizarin Red S (ARS) and oil red O (ORO) staining of BMSCs

After 7, 14, and 28 days of osteogenic induction, the experimental BMSC group and control group were stained for ALP and with 0.2% ARS and ORO following the manufacturer's protocols. An inverted microscope was used to observe and photograph the cells.

Modeling in rats with hyperlipidemia

26 male Wistar rats in 4-week, feed of high-fat for 8 weeks. TC, TG, HDL, LDL proved successfully in high-lipid model. Experimental group Sdccag3-knockdown, Sdccag3-knockdown as well as control group LV16, LV17 virus were injected into muscle. Planting nails were implanted in the femoral backbone epiphyseal, and materials were taken after 14 days. micro-CT shooting was performed and analyzed, and tissue sections were stained with HE and ORO.

Results:

High fat environment influences BMSCs differentiation

After 7d(Fig.1a) and 14d(Fig.1b) high fat osteogenesis induction, the expression of Sdccag3, LRP5, LRP6, β-catenin, Wnt5a and Wnt5b, osteogenic differentiation indexes ALP and Runx2 in BMSCs decreased, and lipid differentiation indexes FABP4 and PPAR-γ were increased. Meanwhile, the expression of Sdccag3 and LRP5 protein was decreased by immunofluorescence compared with normal osteogenic induction group. In the high lipid group, the formation of mineralized nodules and the activity of ALP decreased, the number of lipid droplets formed increased.Western blot analysis showed (Fig. 1c) that the protein expression of Sdccag3 and osteogenic associated protein Runx2 in the high fat group was significantly lower than that in the normal group,and Lipid-related protein PPAR-γ was significantly higher. This suggests that the high-fat environment inhibits the osteogenic differentiation of BMSCs and enhance the adipogenic differentiation of BMSCs.

After 7d,14d and 28d osteogenesis induction under normal/high lipid conditions, ALP, ORO and ARS were conducted. As is shown in Fig.1d-f: After ALP staining, the precipitation of gry-black granules in the cytoplasm of BMSCs in the high fat group was significantly less than that in the normal group, indicating that the osteogenic differentiation ability of BMSCs in the high fat group was significantly weakened compared with the normal group; Fat droplets of different sizes, round like and red stains were observed in BMSCs of high fat group, while no fat droplets were observed in BMSCs of normal group. These results indicated that the stimulation of high lipid environment increased the adipogenic differentiation of BMSCs; Compared with the normal group, the number of mineralized nodules in the high fat group was significantly reduced, indicating that the osteogenic differentiation ability of BMSCs was inhibited by the high fat environment.

After 7 days of osteogenic induction under normal/high fat conditions, immunofluorescence staining results showed (Fig.1g) that Sdccag3 protein expression was higher in BMSCs in normal group, and significantly decreased in BMSCs in high fat group. In addition, compared with the normal group, The expression of LRP5 protein in BMSCs of high fat group was significantly decreased. The expression changes of Sdccag3 and LRP5 were consistent in normal group and high lipid group.

High lipid environment inhibits the osteogenic differentiation of BMSCs, and the tendency of osteogenic differentiation was weakened. Meanwhile, it promotes adipogenic differentiation and increases fat

formation. The mRNA and the protein level of related molecules in the Wnt signaling pathway was significantly changed. All these results indicate that high fat environment may affect the osteogenic and lipid differentiation of BMSCs through Wnt signaling pathways.

Low/overexpression of Sdccag3 regulates the osteogenic and lipid differentiation of BMSCs

As is shown in Fig.2a, in 7 days of high fat osteogenesis induction, Sdccag3 expression level in BMSCs inhibited Sdccag3 group was significantly lower than that in LV16 group, and the expressions of LRP5, LRP6, β-catenin, Wnt5a and Wnt5b, Runx2 and ALP were also significantly decreased. After overexpressed Sdccag3, the expression level of Sdccag3 was significantly higher than that of LV17 group, so were the expressions of LRP5, LRP6, β-catenin, Wnt5a, Wnt5b, Runx2 and ALP.

As Western Blot analysis show (Fig.2b), the expression of protein levels of Sdccag3 and Runx2 were significantly decreased in Sdccag3-knockdown group. The protein expression levels of Sdccag3 and Runx2 were significantly increased in Sdccag3-overexpression group. This suggests that Sdccag3 is upregulated in BMSCs under high-fat environment by regulating both classical and non-classical Wnt. The expression of related molecules in the pathway promotes osteogenic differentiation, whereas the inhibition of Sdccag3 expression aggravates the barrier to osteogenic differentiation.

After 7 days of high fat osteogenic induction, the gray-black granule precipitations in the cytoplasm of BMSCs with Sdccxag3-knockdown staining were significantly lower than those in the negative control LV16 group. In Sdccag3-overexpression group, the gray and black particle precipitates in the cytoplasm after ALP staining were significantly more than those in LV17 group (Fig.2c). These results indicated that the osteogenic differentiation ability of BMSCs in Sdccag3-knockdown group was significantly inhibited, thus affecting the formation of osteoblasts. Sdccag3-overexpression significantly promoted the osteogenic differentiation ability of BMSCs.

After 28 days of high-fat osteogenic induction, the number of red-stained mineralized nodules in Sdccag3-knockdown group was significantly lower than that in LV16 group, indicating that its osteogenic differentiation effect was weakened. Compared with LV17 group, Sdccag3-overexpression group significantly increased the number of red stained mineralized nodules and enhanced osteogenic differentiation. (Fig.2d)

RT-PCR analysis showed (Fig.2e) that Sdccag3-knockdown mRNA expression of PPAR-γ was significantly increased in BMSCs after 7 days of high-fat osteogenesis induction compared with the negative control LV16 group. Compared with LV17 group, the mRNA expression of PPAR-γ in Sdccag3-overexpression group was significantly decreased.

Western Blot analysis showed (Fig.2f) that Sdccag3-knockdown protein expression of PPAR-γ was significantly increased compared with the corresponding negative control group. In contrast, Sdccag3-overexpression of PPAR-γ was significantly reduced. Those results indicated that Sdccag3 in BMSCs under high lipid environment not only affected the osteogenic differentiation ability of BMSCs, but also

had a certain influence on lipid differentiation. Sdccag3 promotes the osteogenic differentiation of BMSCs, inhibits the lipid differentiation of BMSCs, and plays a role in the homeostasis of BMSCs osteogenic and lipid differentiation.

After 14 days of high fat osteogenic induction, the number of red stained lipid droplets in sdccag3-knockdown group was significantly higher than that in LV16 group, and the lipid droplets were larger and more formed. On the contrary, compared with the control LV17 group, the number of red-stained lipid droplets in Sdccag3-overexpression group was significantly reduced, and the lipid droplets were smaller, indicating that its lipid differentiation ability was weakened. (Fig.2g)

In conclusion, after we osteogenesis induced BMSCs, the expression of Sdccag3 and LRP5, LRP6 were downregulated. The expression of β-catenin, Wnt5a and Wnt5b inhibited the expression of osteogenic differentiation indicators ALP and Runx2,and the expression of Lipid differentiation indicators FABP4 and PPAR-γ was promoted.The over-expression of Sdccag3 promoted the osteogenic differentiation of BMSCs and inhibited the tendency of BMSCs to adipogenic differentiation. Sdccag3 inhibited the osteogenic differentiation of BMSCs and promoted the adipogenic differentiation of BMSCs.

Hyperlipidemia inhibits osteogenesis and promotes lipid formation around implants

All rats in the high-fat group were fed the high-fat diet for 8 weeks, and serological and biochemical tests showed (Table 1) that the serum levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride in the high-fat group (TG) level was significantly increased by 2 times or more, and high-density lipoprotein cholesterol (HDL-C) was significantly decreased. This indicated that the hyperlipidemia rat model was successfully established.

Table 1: Serum lipid level of normal group and high lipid group (mean±SD, unit: mmol/L)

	Normal group	HF group
TC	1.373±0.253	2.613±0.123*
TG	1.257±0.645	4.383±1.182*
HDL	1.257±0.645	0.993±0.070*
LDL	0.880±0.078	0.677±0.174*

* There was significant difference between normal group and high fat group (P<0.05).

4 weeks after the implantation of bilateral femoral implants in rats, RT-PCR analysis showed (FIG. 3a,b) that compared with the normal group, the expressions of Sdccag3, Runx2 and ALP in the bone tissues around implants in the high-fat group were significantly decreased. Meanwhile, the expressions of LRP5, LRP6, β-catenin, Wnt5a and Wnt5b were also significantly decreased. Compared with normal group, the expression of lipid differentiation indexes FABP4 and PPAR-γ in high fat group was significantly increased

Micro-CT scanning and 3D reconstruction results (Fig.3c) showed that the new bone trabecular formation around implants in the high-fat group was significantly less than that in the normal group, and the formation of new bone was significantly reduced, indicating that hyperlipidemia had a significant inhibitory effect on osteogenesis around implants in rats. Bone morphometric analysis results (Fig.3d-e) showed that BV/TV in high fat group was significantly lower than that in normal group. The bone structure parameters of Tb. N and Tb. Th were decreased, while Tb. Sp was increased, and the differences were statistically significant compared with normal group. This indicated that osteogenesis around implants was significantly inhibited in the high-fat group, thus affecting the formation of new bone around implants.

Oil-red O staining results of hard tissue sections showed (Fig.3f) that there was almost no red-stained fat in the femoral bone tissue of rats in the normal group, while more red-stained fat was found in the femur of rats in the high-fat group.

In conclusion, in hyperlipidemia rat model, osteogenic differentiation of bone tissues around implants was inhibited and bone formation was reduced. The adipogenic differentiation and adipogenesis of bone tissue around implant were enhanced. In Bone tissue around implants in hyperlipidemia rats, the expression of Sdccag3 was down-regulated, while the expression of Wnt signaling pathway marker genes LRP5, LRP6, β-catenin and Wnt5a and Wnt5b were down-regulated.

Sdccag3 influenced bone and fat formation around implants

4 weeks after the implantation of bilateral femoral implants in rats, RT-PCR analysis showed (Fig. 4a) that the expression level of Sdccag3 in the Sdccag3-knockdown group was significantly lower than that in the LV16 group, the expressions of Runx2 and ALP were significantly decreased, while LRP5, LRP6, β-catenin, Wnt5a and Wnt5b were also significantly decreased. However, it turns out just the opposite in Sdccag3-overexpression group.

These results suggest that in hyperlipidemia rats, the overexpression of Sdccag3 may promote the formation of new bone through the classical and non-classical Wnt pathways, and the decrease of Sdccag3 may also cause the obstacle of new bone formation through the same pathways.

As is shown in Fig.4b, in Sdccag3-knockdown group, the expression of FABP4 and PPAR-γ in the bone tissues around implants of hyperlipidemia rats was significantly increased. On the contrary, the expressions of FABP4 and PPAR-γ were significantly decreased in sdccag3-overexpression group.

These results indicated that the increase of Sdccag3 inhibited the adipogenic differentiation of bone tissue while promoting the osteogenesis around implants in hyperlipidemia rats, while the decrease of Sdccag3 inhibited the osteogenesis around implants and promoted the adipogenic differentiation of bone tissue. The results showed that Sdccag3 plays a certain role in osteogenesis and lipid differentiation of peri-implant bone tissue in hyperlipidemia rats.

In Sdccag3-knockdown group, the new bone trabecula around implants was significantly less than that in LV16 group. In the Sdccag3-overexpression group, compared with the negative control LV17 group, new bone trabeculae around implants were significantly increased. Those results suggest that the overexpression of Sdccag3 pairs can significantly promote osteogenesis around implants in hyperlipidemia rats. (Fig.4c)

BV/TV in Sdccag3-knockdown group was significantly lower than that in LV16 group. Bone structure parameters Tb. N, Tb. Th were higher, and Tb. Sp was lower. In Sdccag3-overexpression group, the results are just the reverse. This indicated that the formation of new bone around implants was significantly inhibited in the Sgccag3-knockdown group, thus affecting the implant-bone binding, while the Sdccag3-overexpression group significantly promoted the formation of new bone around implants and improved the implant-bone binding. Fig.4d-e

As shows in the Fig.4f, compared with LV16, the Sdccag3-knockdown group significantly reduced the formation of new bone around implants, the number of trabecular bone and bone mass, and the osteogenesis around implants. On the contrary, in the Sdccag3-overexpression group, we can find different results. Those results suggest that the upregulation of Sdccag3 can enhance new bone formation around implants in hyperlipidemia rats.

Oil red O staining results of hard tissue sections (Fig.4g) showed that, compared with LV16 group, the red-stained fat in the femur of Sdccag3-knockdown group was significantly increased; On the contrary, compared with LV17 group, the formation of intra femur fat was significantly reduced in Sdccag3-overexpression group. This suggests that the decrease of Sdccag3 promotes adipogenesis, while the increase of Sdccag3 inhibits adipogenesis.

In all, the upregulation of Sdccag3 promoted osteogenesis and inhibited lipid formation. The downregulation of Sdccag3 inhibits osteogenesis and promotes lipid formation. Sdccag3 was positively correlated with the expression of Wnt signaling pathway marker genes LRP5, LRP6, β-catenin, Wnt5a, Wnt5b.

Discussion:

Bone marrow mesenchymal stem cells (BMSCs) can differentiate into competing lineages, including osteogenic and lipid pathways. One of the keys to controlling the interrelationship between osteogenic and lipid-forming lineages is Wnt signaling, which stimulates osteogenesis and inhibits lipid-forming. Activation of Wnt pathway is critical for inducing osteoblast differentiation [13]. Recent studies have

shown that Wnt signaling also plays an important role in adipogenesis and differentiation of BMSC into mature adipocytes. Autocrine Wnt expression has been shown to inhibit terminal differentiation from prebody adipocytes to mature adipocytes [14.15]. It has been found that PPAR-γ plays a critical role in maintaining bone homeostasis in animals and humans. In animal models, decreased PPAR-γ activity resulted in increased bone mass and osteoblast number while increased PPAR-γ activity resulted in a significant decrease in bone mass and changes in bone microstructure. PPAR-γ was found to interact in tandem with Wnt /β-catenin in an opposite way [16]. In this study, it was found that the osteogenic differentiation ability of BMSCs was inhibited while the lipid differentiation was enhanced under high lipid environment. At the molecular biological level, the mRNA protein expression level of related molecules in the Wnt signaling pathway in BMSCs induced by high lipid osteogenesis was significantly changed. These include LRP5, LRP6 and β-catenin in classical Wnt signaling pathways, Wnt5a and Wnt5b in non-classical Wnt signaling pathways, osteogenic differentiation indicators ALP and Runx2, lipid differentiation indicators FABP4 and PPAR-γ. In addition, morphology showed that the staining positive degree of alizarin red and ALP staining of BMSCs induced by high fat osteogenesis was significantly lower than that of the control group, while the number of lipid droplets formed in oil red O staining was significantly higher than that in the control group. These results indicate that the stimulation of high fat environment may affect the osteogenic and lipid differentiation of BMSCs.

Sdccag3 is a 45 kD protein with a c-terminal coiled helical domain. It has been shown to localize early and recirculating endosomes and interact with important components of the Retromer complex for membrane receptor sequencing. In addition, Sdccag3 also locates in the primary cilia and interacts with the flagellate transporter 88, which plays an important role in cilia genesis, formation and cargo localization in the primary cilia. Subsequently, proteins that modulate sensitivity to TNF - induced apoptosis were identified during screening [17–20]. In this experiment, Sdccag3 was confirmed to promote osteogenesis and inhibit lipid formation from molecular biology and morphology levels by inhibiting or overexpressing Sdccag3 in BMSCs. The expressions of LRP5, LRP6, β-catenin and Wnt5a, Wnt5b were positively correlated with the expression of Sdccag3. This is of great significance for exploring the pathway mechanism of the functional role of Sdccag3 in BMSCs osteogenesis and lipid differentiation, while the specific ways of Sdccag3 in promoting osteogenesis and inhibiting lipid formation need further study.

With the development of research, hyperlipidemia has been considered as a risk factor for more and more diseases. There is evidence that bone tissue may change due to elevated triglyceride and cholesterol levels, and excessive LDL cholesterol with dyslipidemia can lead to poor bone metabolism or bone integration around dental implants [21]. In addition, hyperlipidemia, increased LDL-C and TG levels, and decreased HDL-C levels may lead to the risk of peri-implant inflammation [22]. In addition, dyslipidemia has been shown to impair bone parathyroid hormone synthesis and metabolism [23]. Those evidence suggest that dyslipidemia is associated with abnormal bone mass. This study also found that in hyperlipidemia rats, peripheral osteogenesis of implants was inhibited. In the hyperlipidemia animal model, the amount of new bone around implants decreased, bone fat formed around implants, and fat

accumulated in bone marrow. This may be related to the imbalance of osteogenic and adipogenic differentiation of BMSCs.Wnt5a and Wnt5b have been reported to have certain anti-adipogenesis ability, and their function is based on kinase-mediated PPAR-γ to affect adipogenesis, but their role in adipogenic differentiation is still controversial. In this experiment, the expression of Wnt5a and Wnt5b was increased in BMSCs with reduced adipogenic differentiation ability, while the expression of Wnt5a and Wnt5b was decreased in BMSCs with enhanced adipogenic differentiation ability.

In this study, it was found that the upregulation of Sdccag3 not only promoted osteogenesis around the implant in hyperlipidemia rats, but also had a certain inhibitory effect on the adipose formation of bone tissue, showing a certain saving effect on the bone environment under the condition of high fat. In addition, mRNA expressions of Wnt co-receptors LRP5, LRP6 and β-catenin were up-regulated in classical Wnt pathway, and mRNA expressions of Wnt5a and Wnt5b were up-regulated in non-classical Wnt pathway. The mRNA expressions of ALP and Runx2 were up-regulated, suggesting that Sdccag3 may affect the metabolic process of bone formation through both classical and non-classical Wnt signaling pathways, while the mRNA expressions of FABP4 and PPAR-γ were significantly decreased. In contrast, the downregulation of Sdccag3 showed the opposite result. These results indicate that Sdccag3 has different effects on osteogenic and lipid differentiation of bone.

In addition, through human intervention of Sdccag3 expression, it was found that the expression of LRP5 changed with Sdccag3, and there was a positive correlation between LRP5 and Sdccag3 expression, suggesting that the pathway mechanism of Sdccag3 function LRP5 may be an important target. However, the expression of Sdccag3 was decreased in peri-implant bone tissues of hyperlipidemia rats with low expression of LRP5, suggesting the interaction between Sdccag3 and LRP5. Whether there is a direct interaction between Sdccag3 and LRP5, and whether they are the intermediate targets for Wnt signaling pathway to regulate osteogenic and lipid differentiation, will be the next problem and research target to be solved in this experiment.

Abbreviations

BMSCs
Bone Marrow Mesenchymal Stem Cells
Sdccag3
Serologically defined colon cancer antigen3
LRP5
Low Density Lipoprotein receptor-related protein 5

Declarations

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Availability of data and material:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Contribution:

MQY: Contributed to conception and design, data acquisition and interpretation, performed all statistical analyses, drafted and critically revised the manuscript.

FLH: Contributed to conception and design, data analysis and interpretation, drafted and critically revised the manuscript.

HPR: Contributed to conception and design, data acquisition and analysis, drafted the manuscript.

QSG: Contributed to design, data acquisition.

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Ethics approval and consent to participate:

The animal experiment has been approved and recognized by the Ethics Committee of Shandong University (No. GD201817). This experimental animal was purchased from Jinan Zhangqiu Animal Center. Specific pathogen-free (SPF) Wistar rats were provided by the

Experimental Animal Center of Shandong University (Jinan, China).

Consent for publication:

Not applicable.

Competing interests:

The authors have declared that no conflict of interest exists.

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Figures

Figure 1

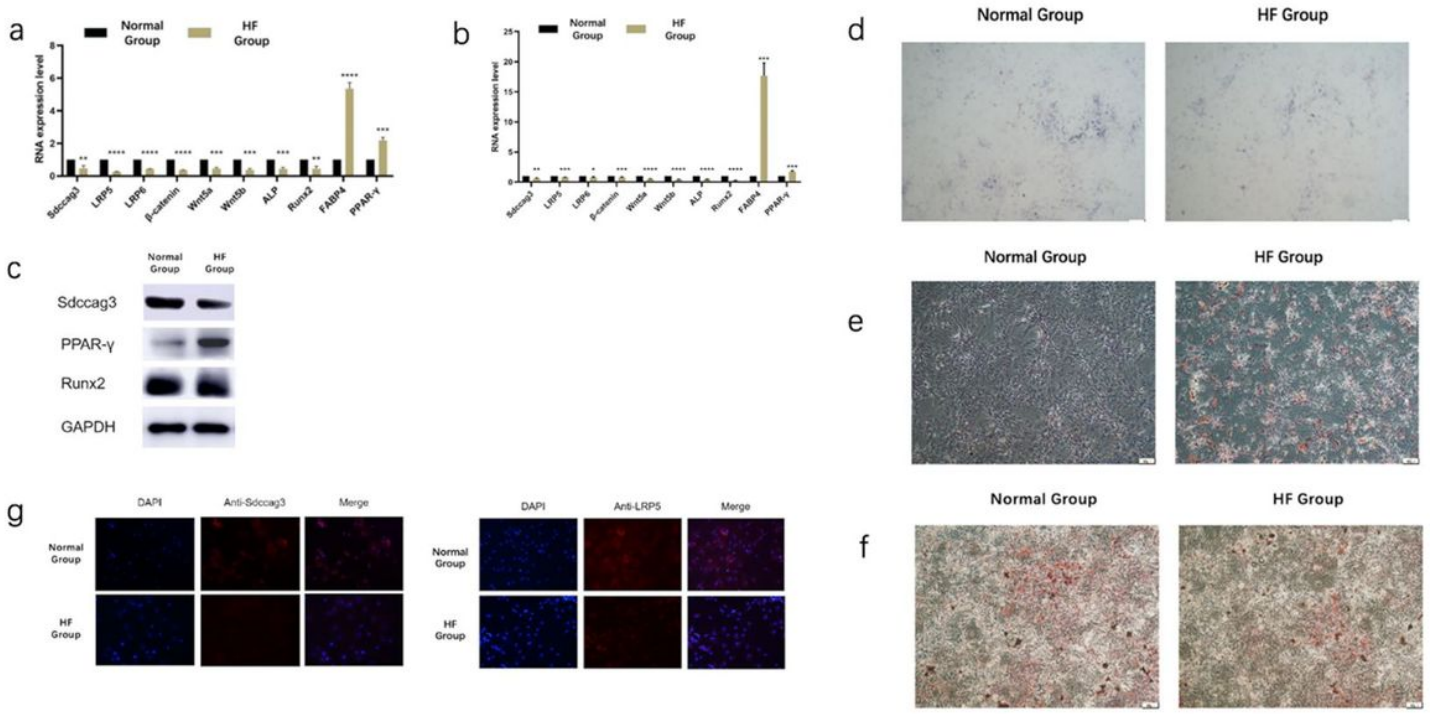

Figure 1

a: RT-PCR analysis of related gene expression of BMSCs in normal group and high fat group after 7 days of osteogenesis induction (**P<0.01, ***P<0.001, ****P<0.0001);b: Rt-PCR was used to detect related gene expression of BMSCs in normal group and high fat group after 14 days of osteogenesis induction. (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001);c: Western Blot analysis of related protein expression of BMSCs in normal group and high fat group after 7 days of osteogenic induction; d: ALP staining results of BMSCs in normal group and high fat group after 7 days of osteogenesis induction; e: Oil red O staining results of BMSCs in normal group and high fat group after 14 days of osteogenesis induction; f: Alizarin red staining results of BMSCs in normal group and high fat group after 28 days of osteogenic induction; g: Immunofluorescence staining results of BMSCs in normal group and high fat group after 7 days of osteogenesis induction.BMSCs: Bone Marrow Mesenchymal Stem Cells

Figure 2

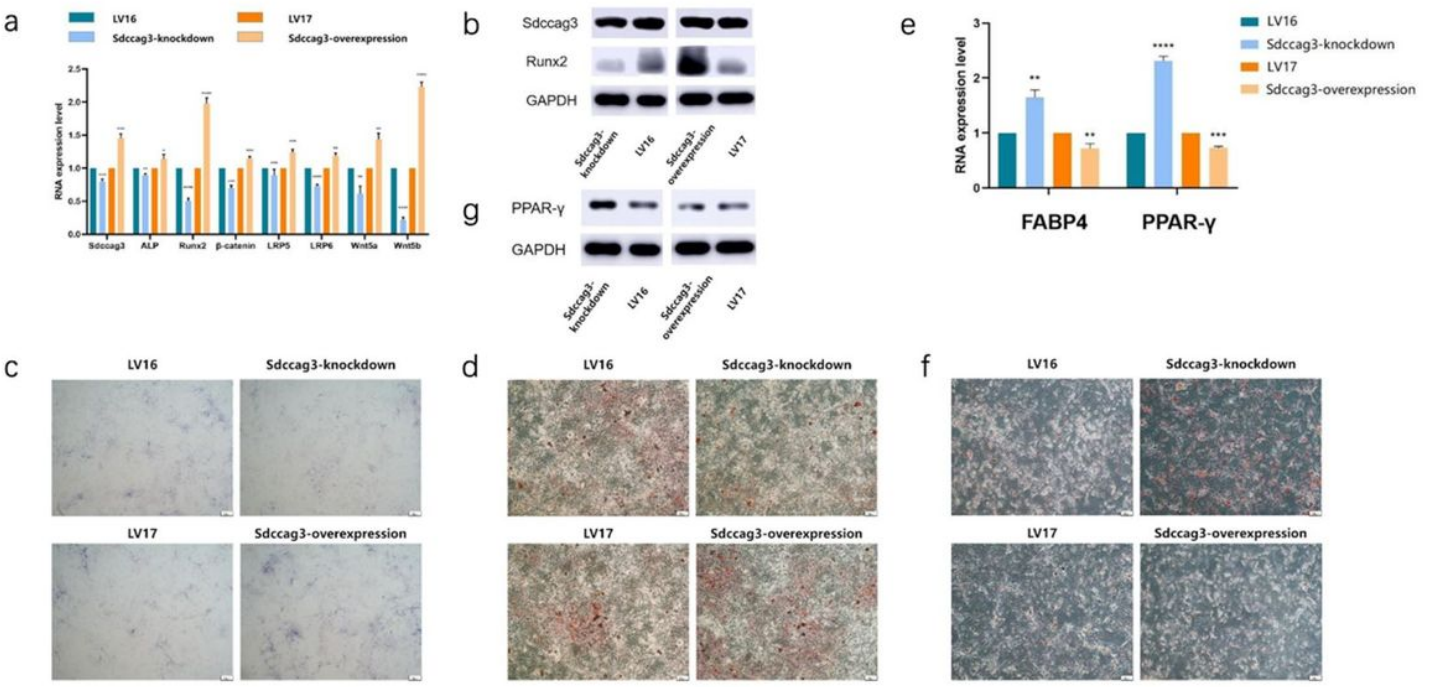

Figure 2

a: (Sdccag3-knockdown: Sdccag3 inhibition table reached the group; LV16: control group; Sdccag3-overexpression: Sdccag3 overexpression group; LV17: negative control group). The expression analysis results of related genes detected by RT-PCR after 7 days of high fat osteogenesis induction(*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001);b: After 7 days of high fat osteogenesis, Western Blot was used to detect related protein expression; c: ALP staining results after 7 days of high fat osteogenesis induction; d: Alizarin red staining after 28 days of high fat osteogenesis induction; e: Rt-PCR was performed after 7 days of high fat osteogenesis induction, and related gene expression analysis(* P < 0.05, * * P < 0.01, * * * P < 0.001, * * * * P < 0.0001);f: Results of oil red O staining after 14 days of high fat osteogenesis induction; g: After 7 days of high fat osteogenesis, Western Blot was used to detect protein expression of PPAR-γ.(Sdccag3: serologically defined colon cancer antigen3 ;BMSCs: Bone Marrow Mesenchymal Stem Cells)

Figure 3

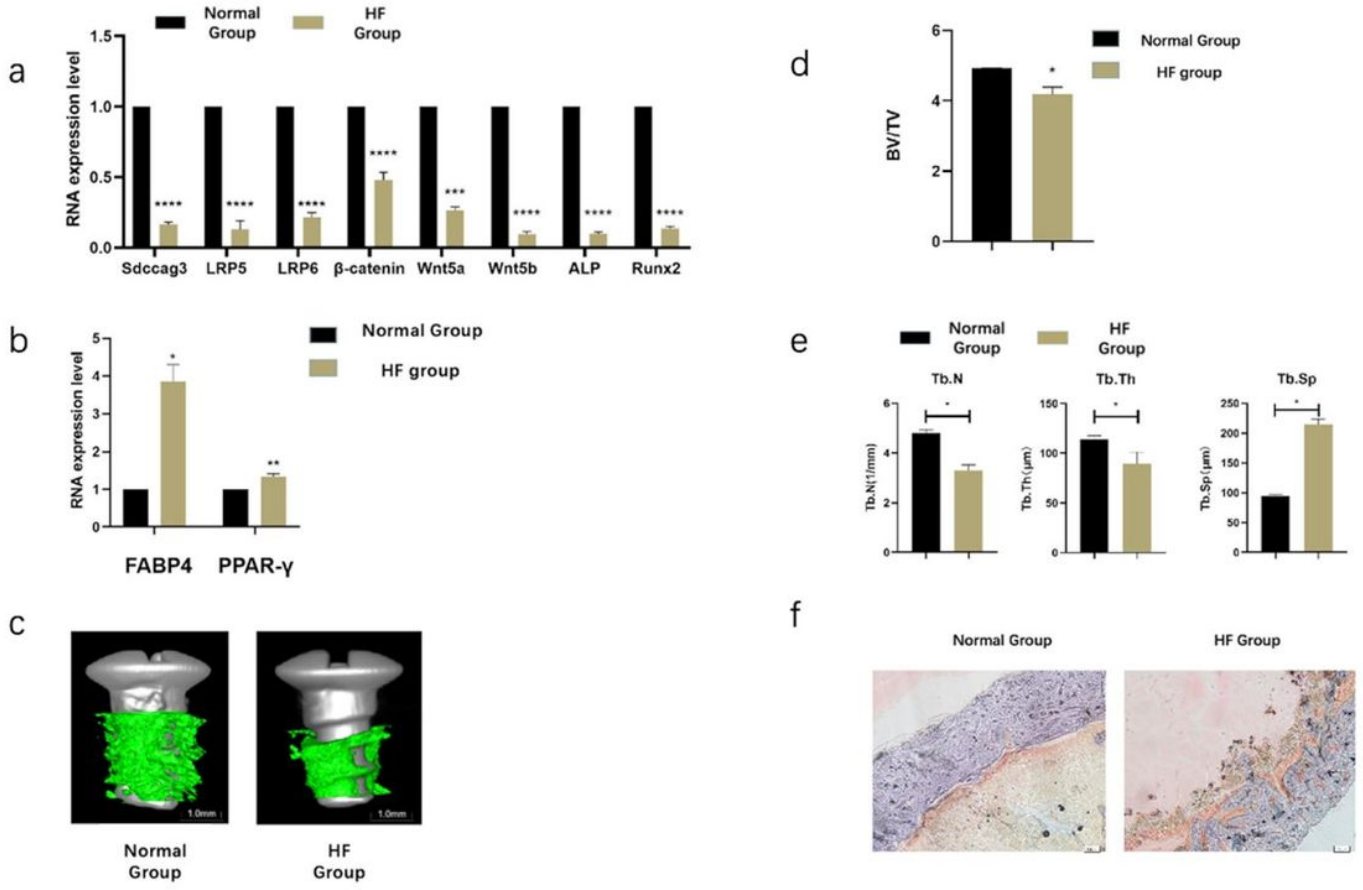

Figure 3

a: RT-PCR analysis of related gene expression in bone tissues around implants of rats in normal group and high fat group(*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001);b: RT-PCR analysis of related gene expression in bone tissues around implants of rats in normal group and high fat group(* P < 0.05, * *P < 0.01);c: After 4 weeks of implantation of bilateral femoral implants in the normal group and the high-fat group, 3d reconstruction images after micro-CT scanning were obtained; d Micro-CT bone morphometric BV/TV analysis of rats in normal group and high fat group, *P<0.05; e: Tb. N, Tb. Th, Tb. Sp of rats in normal group and high fat group were analyzed by micro-CT, *P<0.05; f: Oil red O staining results of hard tissue sections of femur of rats in normal group and high fat group.

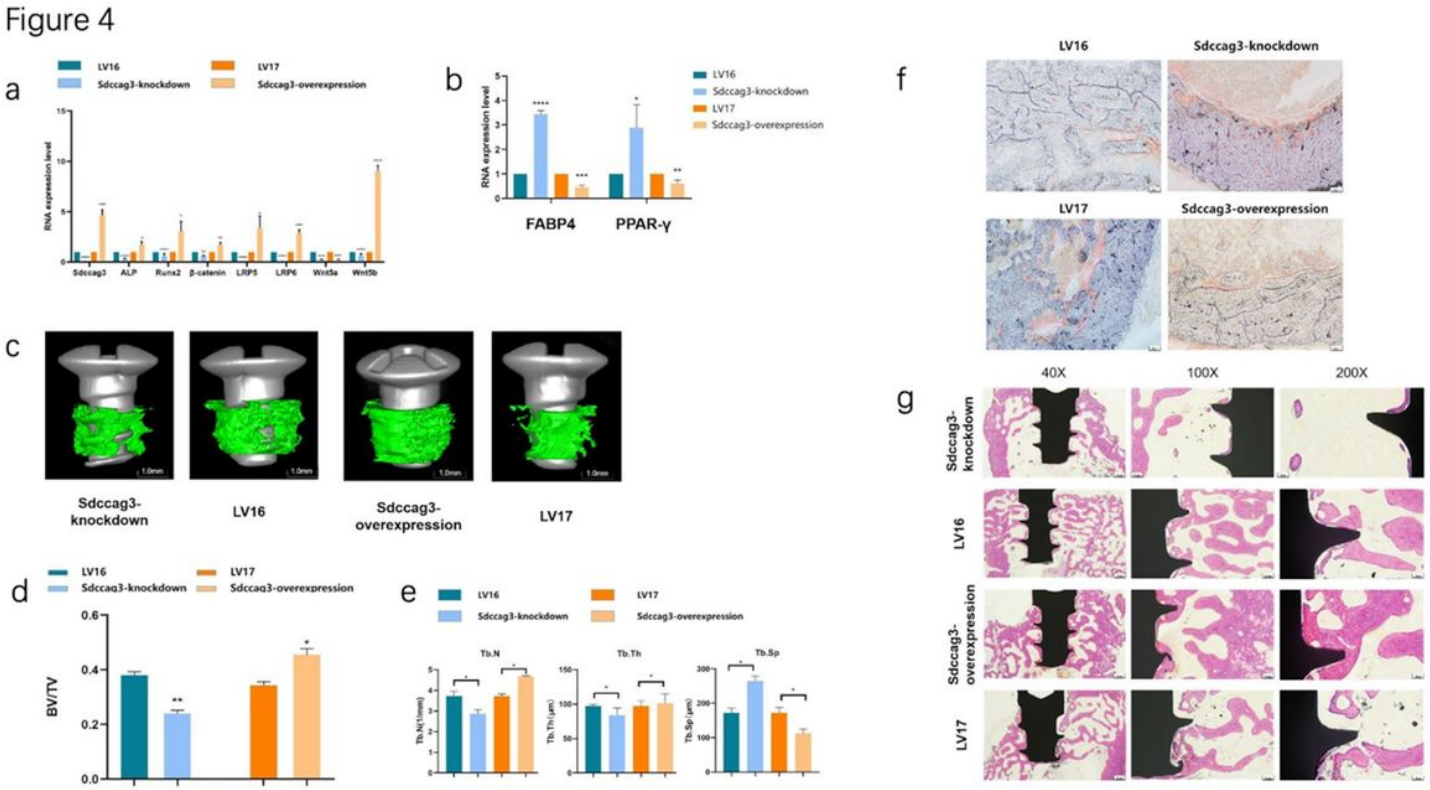

Figure 4

a/b: RT-PCR analysis of related gene expression in bone tissues around implants (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001); c: Micro-CT 3d reconstruction of bilateral femoral implants 4 weeks after implantation; d: Micro-CT bone morphology measurement BV/TV analysis results (*P<0.05, **P<0.01); e: Micro-CT bone morphology measurement Tb. N, Tb. Th, Tb. Sp; f: Results of oil red O staining in hard tissue section of femur; g: HE staining of hard tissue sections after 4 weeks of bilateral femoral implants. 40 x: 40 times mirror; 100X: 100 times mirror; 200X: 200 times.

Supplementary Files

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