Cellular Physiology and Biochemistry

Cell Physiol Biochem 2018;50:1916-1928
DOI: 10.1159/000494872
Published online: 3 November 2018
Accepted: 26 October 2018

Original Paper

GOLM1 Stimulation of Glutamine Metabolism Promotes Osteoporosis via Inhibiting Osteogenic Differentiation of BMSCs

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Key Words
Osteoporosis • BMSCs • GOLM1 • mTOR • Glutamine metabolism

Abstract
Background/Aims: Bone marrow mesenchymal stem cells (BMSCs) play an essential role in osteoporosis. However, the molecular mechanisms and the involvement of glutamine metabolism in osteogenic BMSCs differentiation and osteoporosis remain largely unclear. In this study, we investigated the role of Golgi membrane protein 1 (GOLM1) and glutamine metabolism in BMSCs differentiation and osteoporosis. ***Methods:*** Osteogenic differentiation-inducing media (Odi) was used to induce the osteogenic differentiation of BMSCs. The mRNA expression of GOLM1, ALP, Runx2, Osx, BSP and OCN was determined by qRT-PCR assay. Western blot assay was used to analyze GOLM1, p-mTOR, mTOR, p-S6 and S6 abundance in GOLM1 silencing and over-expressed BMSCs. Glutamine uptake, intracellular glutamine, glutamate and α-KG level was detected using indicated Kits. GOLM1 antibody, glutamine metabolism inhibitors EGCG and BPTES were used to treat ovariectomy (OVX)-induced osteoporosis. Bone mineral density and bone volume relative to tissue volume (%) were analyzed by micro-CT. Serum was collected from osteoporosis patients and healthy participants and subjected to GOLM1 determination using ELISA Kit. ***Results:*** GOLM1 expression and glutamine metabolism were suppressed by Odi. GOLM1 blockage or inhibition of glutamine metabolism promoted the osteogenic differentiation of BMSCs induced by Odi. GOLM1 activated glutamine metabolism depending on the mTOR signaling pathway. *In vivo*, GOLM1 antibody or combination of glutamine inhibitor EGCG and BPTES rescued the osteoporosis in an OVX-operated mouse model. Serum GOLM1 level was increased in the patients of osteoporosis compared with healthy people. ***Conclusion:*** GOLM1 stimulates

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glutamine metabolism to suppress the osteogenic differentiation of BMSCs and to promote osteoporosis. Therefore, GOLM1 activation of glutamine metabolism is a potential target for osteoporosis.

Introduction

Osteoporosis is an age-associated musculoskeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with an increased morbidity and socioeconomic burden [1, 2]. Although multiple treatment options, including cell therapy, supplementation of calcium and vitamin D and bisphosphonates, are currently available for osteoporosis patients [3, 4], the effectiveness and clinical outcomes of these strategies are less satisfactory. Therefore, illustrating the pathological and molecular events contributing to osteoporosis progression may help us gain insight into the underlying mechanisms and develop promising therapeutic treatments.

Osteoporosis is a disease resulting from an imbalance of bone homeostasis, which represents an active coupling process that occurs via bone formation and bone resorption [5]. Bone marrow mesenchymal stem cells (BMSCs) are multipotent stem cells that can differentiate into adipocytes, chondrocytes, and osteoblasts [6-8]. These cells play an essential role in regulating bone/marrow homeostasis [9]. Some studies have revealed that abnormal differentiation of BMSCs promotes the development of osteoporosis [10]. A previous study found that autophagy prevented estrogen deficiency-induced osteoporosis through maintaining the function of bone marrow mesenchymal stem cells [11]. Clinical studies have also demonstrated that postmenopausal osteoporotic women exhibit an adipogenic differentiation of BMSCs [1, 12]. In the last decade, some molecules have been found to play an important role in regulating BMSCs functions and differentiation [13, 14]. However, there is still lack of evidence of critical molecular or signaling transduction contributing to the differentiation of BMSCs.

Golgi membrane protein 1 (GOLM1), a type II Golgi transmembrane protein, is involved in regulating protein synthesis and transport. It has been reported to be stimulated during virus infection, including human immunodeficiency virus (HIV) [15], Hepatitis B virus (HBV) and Hepatitis C virus (HCV) [16]. GOLM1 is up-regulated in various cancer types, including Hepatocellular carcinoma (HCC), prostate cancer and lung cancer, and it promotes cancer progression through multiple molecular mechanisms [17-19]. Besides, dysregulation of GOLM1 has been also reported in other diseases, such as Alzheimer's disease [20, 21]. However, the role of GOLM1 in BMSCs and associated osteoporosis remains unknown.

Based on the initial finding that serum GOLM1 was increased in the osteoporosis patients, we hypothesized that GOLM1 might participate in the progression of osteoporosis. Here, we aimed to investigate the role of GOLM1 in BMSCs differentiation and osteoporosis. Further molecular mechanisms and the involvement of glutamine metabolism in GOLM1 regulation of BMSCs were also explored. Our studies may provide novel therapeutic targets for osteoporosis treatment.

Materials and Methods

Ethics statement

All animal experiments were approved by the Ethics and Research Committee of Soochow University. All procedures were performed following the guidelines of Soochow University.

Measurement of Human serum GOLM1

Serum was collected from different-aged healthy participants, osteoporosis patients and age-matched healthy participants for GOLM1 level measurement. Human Golgi membrane protein 1 (GOLM1) ELISA

Kit was from DLDEVELOP (Canada). A written informed consent was received from all the healthy or osteoporosis participants.

Animals

12-week-old female mice were subjected to ovariectomy (OVX) procedure to induce osteoporosis phenotypes. Mice undergoing a sham operation served as control. GOLM1 antibody (Santa Cruz) injection, EGCG (20mg/kg twice a week), BPTES (10mg/kg twice a week) or combined treatment was performed 8 weeks after OVX for 6 weeks. Then the mice were subjected to micro-CT (Siemens AG, Germany) analysis of bone mineral density (BMD) and bone volume relative to tissue volume (BV/TV, %).

Cell culture

BMSCs were isolated and purified from the marrow of femur and tibia of 12-week-old mice and cultured in α-modified essential medium (α-MEM; Gibco), supplemented with 15% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin. The cells were maintained in a 37°C incubator containing 5% CO_2.

Lentivirus-mediated GOLM1 knockdown in BMSCs

Lentivirus vector pLL3.7 was used to knockdown GOLM1 or GDH in BMSCs. The shRNA sequences were as listed in the supplementary materials (For all supplemental material see www.karger.com/10.1159/000494872/). pLL3.7 shRNA and packaging vectors (VSVG, pMDL and REV) were co-transfected into 293T cells. 48-72 hours after transfection, virus supernatants were harvested and filtered through a 0.45 μm filter. The BMSCs reaching 60-80% confluence were infected with the indicated virus in the presence of polybrene (8 mg/ml). The infection rate was determined by green fluorescence.

Lentivirus-mediated GOLM1 over-expression in BMSCs

Lentivirus carrying GOLM1 cDNA (GLOM1-GFP) or empty control (Ctrl-GFP) were infected to BMSCs at a confluence of 60-80% in the presence of polybrene (8 mg/ml). Western blot was used to check the over-expressed efficiency.

Metabolites and GDH activity measurement

The concentrations of glutamine, glutamate and α-KG (α-Ketoglutarate) were determined by Glutamine Assay Kit (BioAssay Systems), Glutamate Assay Kit (Abcam) and α-Ketoglutarate Assay Kit (Sigma), respectively. GDH activity was analyzed using BioVision kit. All the measurements were performed according to the manufacturer's instructions. The level of glutamine, glutamate and α-KG was normalized to cell number.

Total mRNA isolation and quantitative real-time PCR

Total RNA was isolated from BMSCs using Trizol reagent (Invitrogen) following to the manufacturer's protocol. RNA was subjected to reverse transcription reaction for cDNA synthesis by M-MLV reverse transcriptase (Promega). Quantitative real-time PCR was performed on a Bio-rad IQ5 machine to analyze targeted gene expression using TransStart Green qPCR SuperMix (TransGen Biotech). The PCR primers used were listed in the supplementary materials. β-actin serves internal control.

Western blot

Total protein was isolated from BMSCs using lysis buffer (Beyotime) and concentration was measured by BCA protein assay kit (Beyotime). 60μg of total protein was separated on a 12 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidenefluoride membrane (PVDF; Millipore). The membranes were blocked with 5% skim milk for at least 60 minutes at room temperature and incubated with primary antibodies at 4°C overnight. Antibodies against GOLM1, β-actin and all the secondary antibodies were purchased from Santa Cruz. Antibodies against p-mTOR, mTOR, p-S6, S6 were from Cell Signaling Technology.

Statistical analysis

All the results were presented as the mean ± standard deviation (SD) of at least three independent experiments. Student's t-test was used to analyze the statistical significance between two groups. ANOVA

followed by the Bonferroni post hoc test was applied for multiple comparisons when the assumptions (equal variances and normal distribution) were satisfied. P value of less than 0.5 was considered significant.

Results

GOLM1 inhibits the osteogenic differentiation of BMSCs

Firstly, we determined whether GLOM1 was involved in BMSCs differentiation. The expression and activity of alkaline phosphatase (ALP), which is an early differentiation marker, were obviously increased after osteogenic differentiation-inducing media (Odi) treatment (Fig. 1A). During the osteogenic differentiation of BMSCs, both the mRNA and protein level of GOLM1 decreased in Odi-treated BMSCs (Fig. 1A). We then silenced GLOM1 in BMSCs, followed by Odi stimulation. GOLM1 knockdown led to a slightly increased mRNA expression of ALP (Fig. 1B and 1C). Runt-related transcription factor 2 (Runx2) and osterix (Osx), two osteoblast-specific transcription factors and other bone matrix genes, including bone sialoprotein (BSP) and osteocalcin (OCN) were also up-regulated in GOLM1 silencing BMSCs (Fig. 1B and 1C). The difference became bigger when treated with Odi (Fig. 1B and 1C). Consistently, GOLM1 antibody exhibited a comparable effect on osteogenic differentiation of BMSCs (Fig. 1D). In contrast, GOLM1 ectopic expression reduced the osteogenic differentiation of BMSCs, as indicated by down-regulation of ALP, Runx2, Osx, BSP and OCN (Fig. 1E). Collectively, we revealed that GOLM1 suppressed the differentiation of BMSCs into osteoblasts.

GOLM1 activates glutamine metabolism by increasing GDH activity

We next explored whether GOLM1 regulated glutamine metabolism in BMSCs. GOLM1 knockdown BMSCs consumed significantly less glutamine compared with shCtrl BMSCs (Fig. 2A). To test whether GOLM1 regulates glutamine uptake or glutamine conversion, we measured the intracellular level of glutamine, glutamate and α-KG. Glutamine and glutamate in BMSCs were obviously elevated after GOLM1 knockdown (Fig. 2B-2D). GOLM1 over-expressed BMSCs consumed more glutamine, while had decreased intracellular glutamine, glutamate (Fig. 2E-2G). α-KG level was increased in GOLM1 over-expressed BMSCs comparing to Ctrl BMSCs (Fig. 2H). Based on these results, we investigated whether GOLM1 regulated glutamine to glutamate conversion or glutamate to α-KG conversion (Fig. 2K), which are catalyzed by glutaminase (GLS) or dehydrogenase (GDH), respectively. Our results showed that GOLM1 knockdown suppressed GDH activity, while GOLM1 over-expression activated GDH (Fig. 2I and 2J). Taken together, GOLM1 promoted glutamate to α-KG conversion through activating GDH.

mTOR signaling is critical for GOLM1 activation of glutamine metabolism

We then determined whether mTOR signaling participated in GOLM1-mediated glutamine metabolism. mTOR signaling was suppressed by GOLM1 knockdown, as indicated by decreased phosphor-mTOR and phosphor-S6 in shGOLM1 BMSCs compared with shCtrl BMSCs (Fig. 3A and 3B). By contrast, GOLM1 ectopic expression led to increased activity of mTOR (Fig. 3C). Furthermore, we measured GDH activity and glutamine uptake in GLOM1 over-expressed or knockdown BMSCs treated with or without rapamycin. Rapamycin significantly suppressed the GDH activity of GOLM1 over-expressed BMSCs, while had a marginal effect on that of GOLM1 knockdown BMSCs (Fig. 3D and 3E). Consistently, glutamine uptake in GOLM1 over-expressed BMSCs was obviously decreased after rapamycin treatment, whereas it remained unchanged in GOLM1 knockdown BMSCs treated with rapamycin (Fig. 3F and 3G). Our results indicate that GOLM1 activates glutamine metabolism depending on the mTOR signaling pathway.

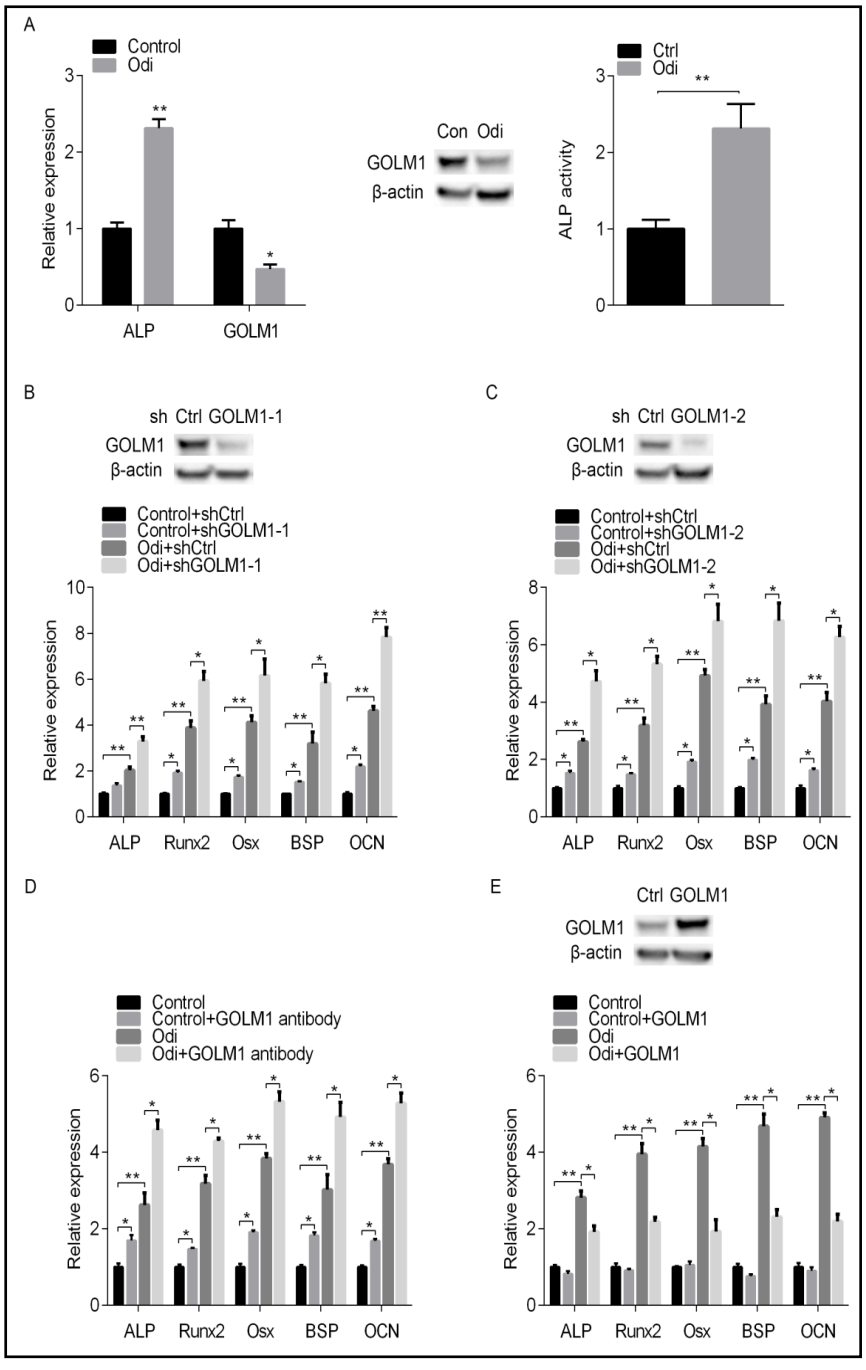

Fig. 1. GOLM1 prevents the osteogenic differentiation of BMSCs. (A) BMSCs treated with or without osteogenic differentiation-inducing media (Odi) were subjected to qRT-PCR analysis of ALP and GOLM1 (Left), western blot analysis of GOLM1 (Middle) and ALP activity measurement (Right). *p<0.05, **p<0.01. (B and C) shCtrl, shGOLM1-1 or shGOLM1-2 BMSCs were treated with or without Odi and then subjected to qRT-PCR analysis of ALP, Runx2, Osx, BSP and OCN. Western blot results showed that GOLM1 was efficiently knocked down in shGOLM1-1 or shGOLM1-2 BMSCs. *p<0.05, **p<0.01. (D) BMSCs pretreated with GOLM1 antibody were immediately treated with or without Odi and then subjected to qRT-PCR analysis of ALP, Runx2, Osx, BSP and OCN. *p<0.05, **p<0.01.(E) Ctrl or GOLM1 over-expressed BMSCs were treated with or without Odi and then subjected to qRT-PCR analysis of ALP, Runx2, Osx, BSP and OCN. Western blot results showed that GOLM1 was over-expressed in GOLM1 BMSCs. *p<0.05, **p<0.01.

Inhibition of glutamine metabolism promotes osteogenic differentiation of BMSCs

We have shown that GOLM1 activates glutamine metabolism and impairs osteogenic differentiation of BMSCs. However, the role of glutamine metabolism in BMSCs differentiation remains unclear. Next, we explored the involvement of glutamine metabolism in BMSCs differentiation based on Odi treatment. Firstly, GDH activity was decreased in Odi BMSCs as compared with Ctrl BMSCs (Fig. 4A). In addition, Odi suppressed the glutamine consumption

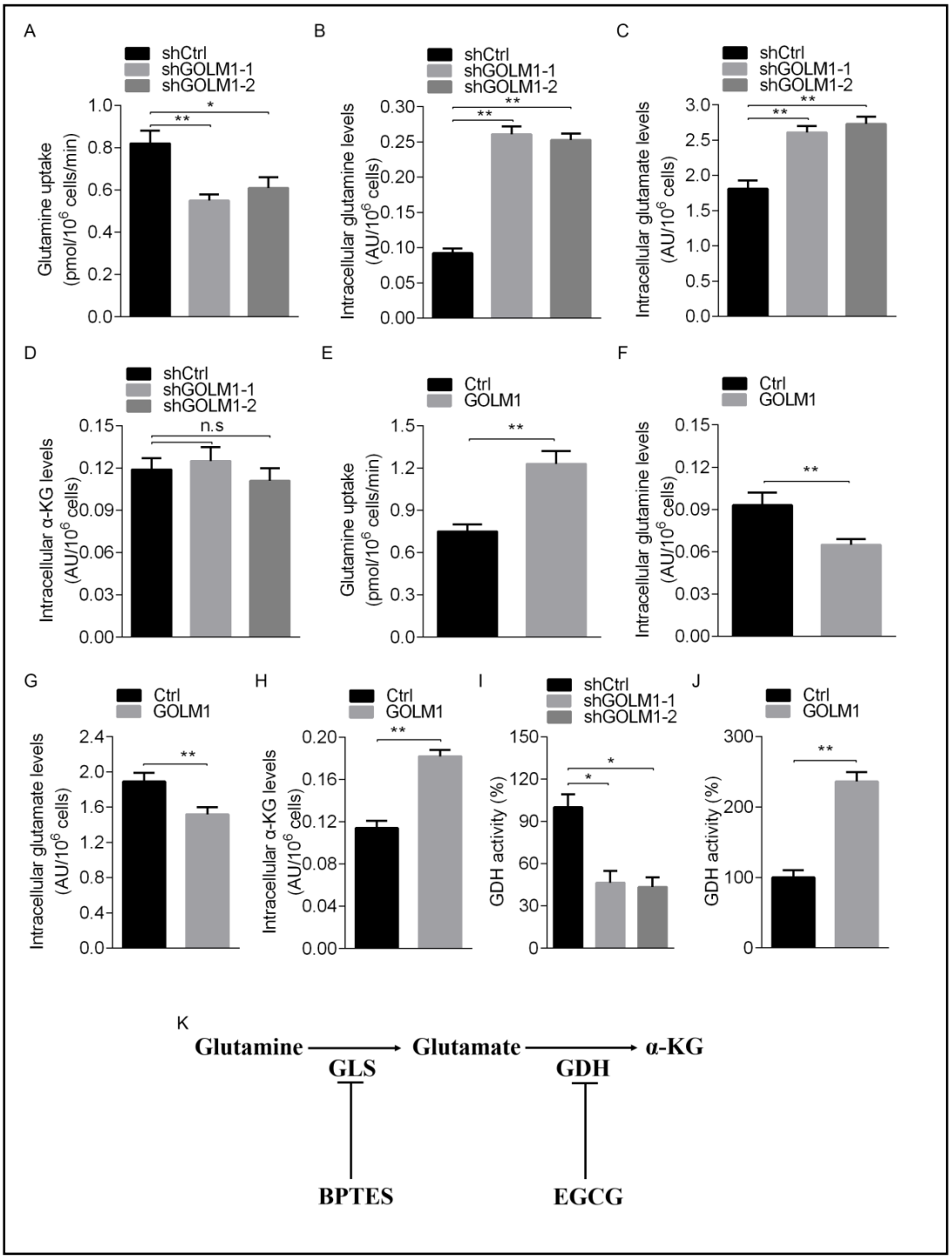

Fig. 2. GOLM1 activates glutamine metabolism in BMSCs.(A) Glutamine uptake in shCtrl, shGOLM1-1 or shGOLM1-2 BMSCs. * p<0.05, ** p<0.01.(B-D) Intracellular glutamine (B), glutamate (C) or α-KG (D) level in shCtrl, shGOLM1-1 or shGOLM1-2 BMSCs. ** p<0.01. n.s, no significance.(E) Glutamine uptake in Ctrl or GOLM1 BMSCs. ** p<0.01.(F-H) Intracellular glutamine (F), glutamate (G) or α-KG (H) level in Ctrl or GOLM1 BMSCs. ** p<0.01.(I) GDH activity of shCtrl, shGOLM1-1 or shGOLM1-2 BMSCs. ** p<0.01.(J) GDH activity of Ctrl or GOLM1 over-expressed BMSCs. ** p<0.01.(K) A diagram of the enzymes involved in glutamine metabolism.

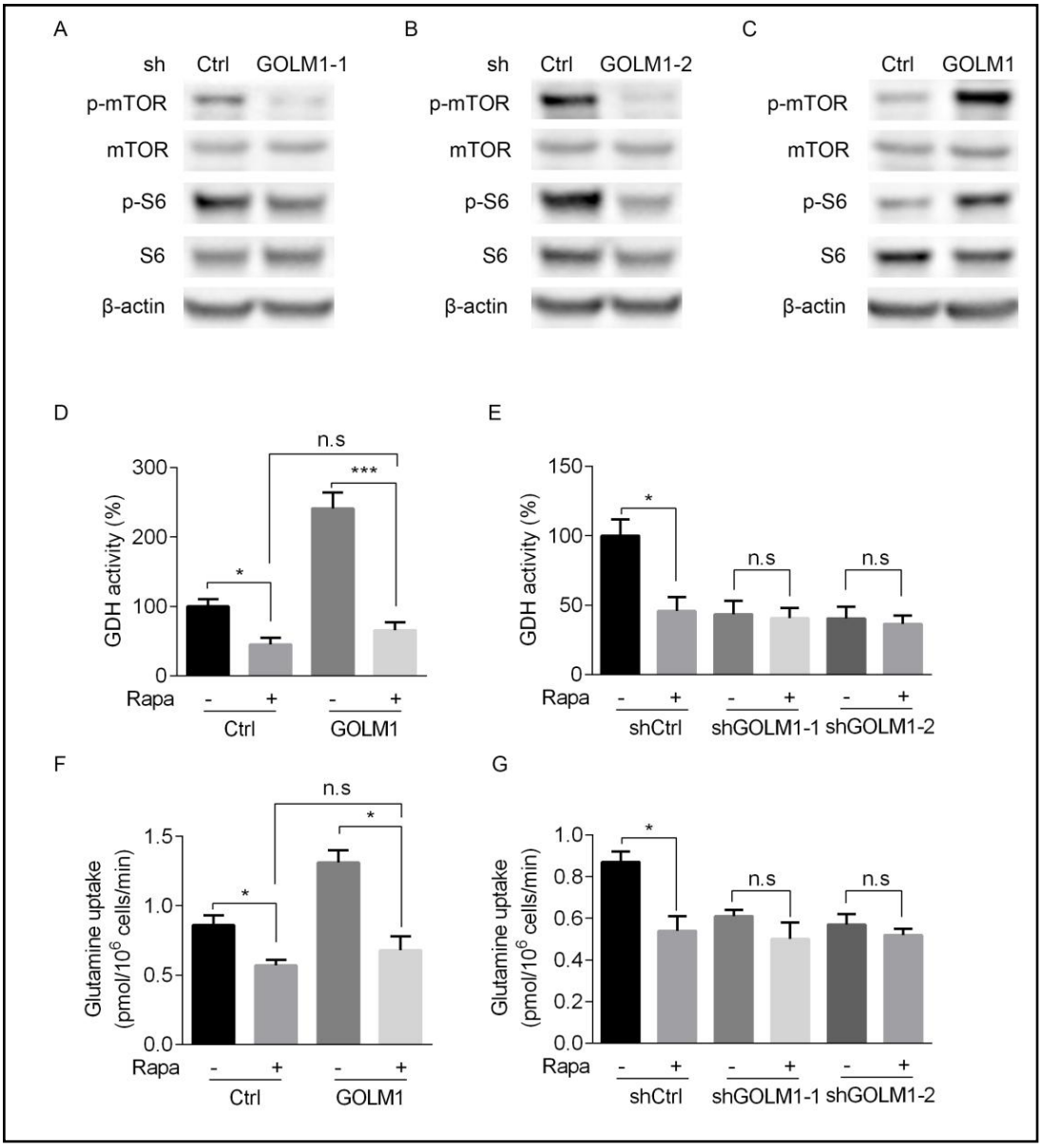

Fig. 3. GOLM1 promotes glutamine metabolism through activation of mTOR signaling pathway. (A and B) Western blot analysis of phosphor-mTOR, mTOR, phosphor-S6, S6 in shCtrl and shGOLM1-1 BMSCs (A) or shCtrl and shGOLM1-2 BMSCs (B). β-actin serves as internal control.(C) Western blot analysis of phosphor-mTOR, mTOR, phosphor-S6, S6 in Ctrl and GOLM1 over-expressed BMSCs. β-actin serves as internal control.(D) Ctrl or GOLM1 over-expressed BMSCs were treated with or without rapamycin (Rapa) and then subjected to measurement of GDH activity. n.s, no significance. *p<0.05, ***p<0.001.(E) shCtrl, shGOLM1-1 or shGOLM1-2 BMSCs were treated with or without rapamycin (Rapa) and then subjected to measurement of GDH activity. n.s, no significance. *p<0.05.(F) Glutamine uptake in Ctrl or GOLM1 over-expressed BMSCs that were treated with or without rapamycin (Rapa). n.s, no significance. *p<0.05.(G) Glutamine uptake in shCtrl, shGOLM1-1 or shGOLM1-2 BMSCs that were treated with or without rapamycin (Rapa). n.s, no significance. *p<0.05.

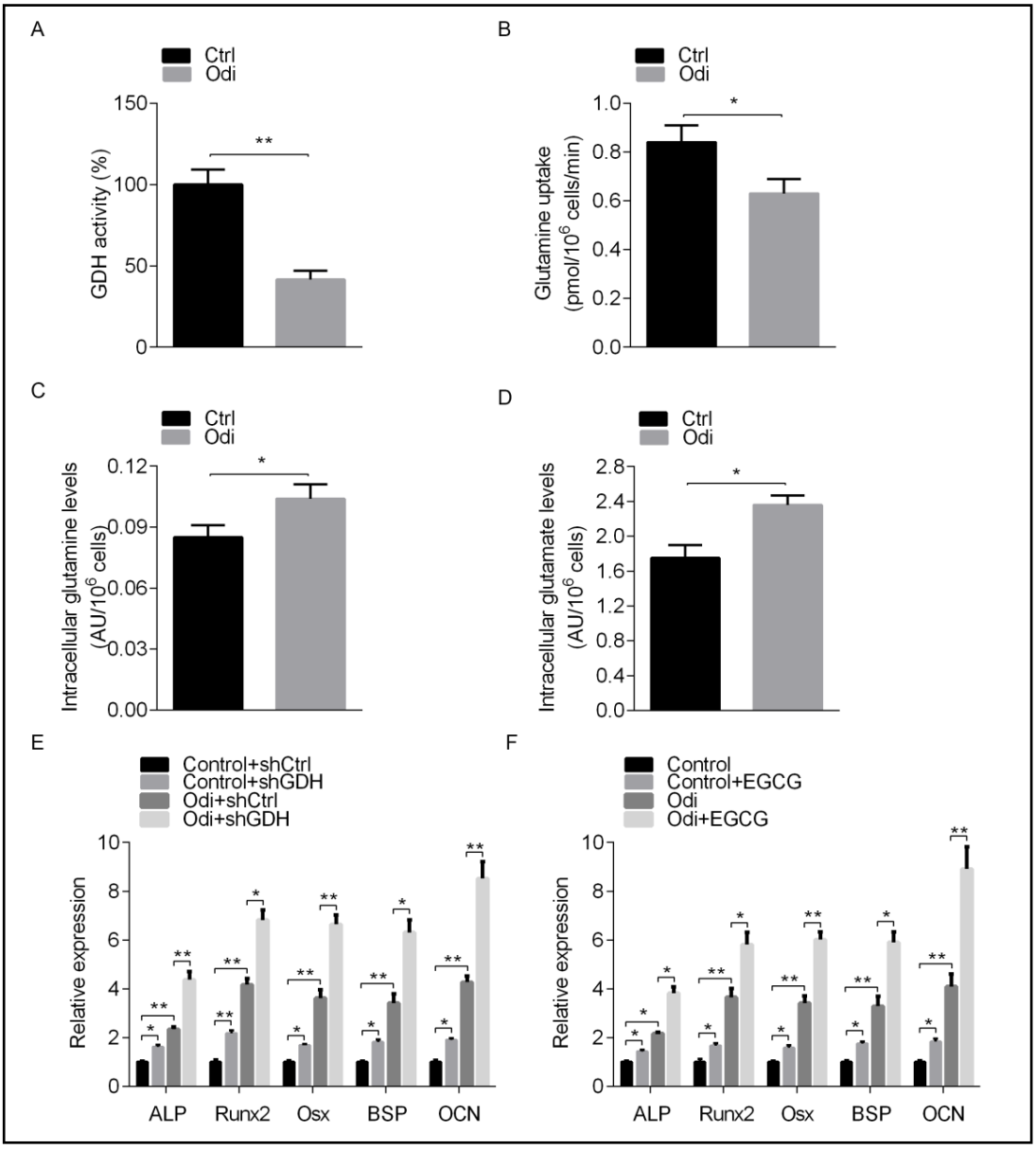

Fig. 4. Suppression of glutamine metabolism promotes the osteogenic differentiation of BMSCs. (A-D) GDH activity (A), glutamine uptake (B), intracellular glutamine level (C) and intracellular glutamate level (D) in BMSCs treated with or without Odi. *p<0.05, **p<0.01. (E) shCtrl or shGDH BMSCs were treated with or without Odi and then subjected to qRT-PCR analysis of ALP, Runx2, Osx, BSP and OCN. *p<0.05, **p<0.01. (F) BMSCs pretreated with EGCG were immediately treated with or without Odi and then subjected to qRT-PCR analysis of ALP, Runx2, Osx, BSP and OCN. *p<0.05, **p<0.01.

in BMSCs (Fig. 4B). Consistent with the findings of GOLM1, intracellular glutamine and glutamate level increased after Odi stimulation (Fig. 4C and 4D). These results suggested that glutamine metabolism might be involved in the osteogenic differentiation of BMSCs. To validate this possibility, we analyzed the role GDH knockdown and glutamate inhibitor EGCG on BMSCs differentiation. Our results showed that GDH knockdown and EGCG slightly promoted the osteogenic differentiation of BMSCs, as indicated by up-regulation of ALP, Runx2, Osx, BSP and OCN (Fig. 4E and 4F). Odi stimulation caused an even more significant up-regulation of these genes (Fig. 4E and 4F). We demonstrated that inhibition of glutamine metabolism was essential to osteogenic differentiation of BMSCs.

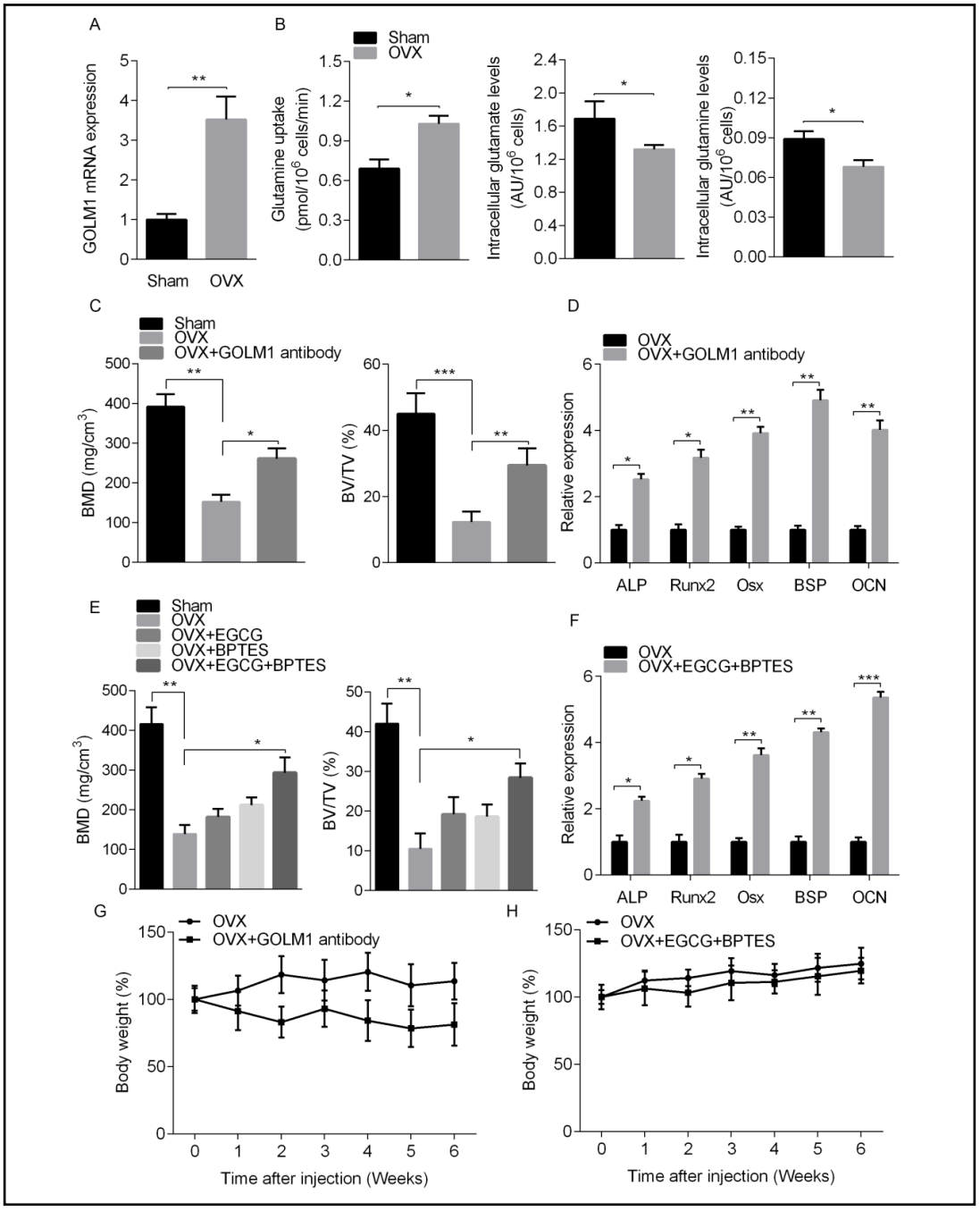

Fig. 5. GOLM1 or GDH knockdown rescues OVX-induced osteoporosis. (A and B) BMSCs isolated from Sham or OVX-operated female mice were subjected to qRT-PCR analysis of GOLM1 and detection of glutamine uptake, intracellular glutamate and glutamine. n=5-6. (C and D) 8 weeks after OVX, the female mice were treated with or without GOLM1 antibody for 6 weeks and then subjected to micro-CT analysis of Bone Mineral Density (BMD) and bone volume relative to tissue volume (BV/TV, %) (C), and qRT-PCR analysis of ALP, Runx2, Osx, BSP and OCN (D) n = 5-6. (E and F) 8 weeks after OVX, the female mice were treated with vehicle, EGCG, BPTES or combination of EGCG and BPTES for 6 weeks and then subjected to micro-CT analysis of Bone Mineral Density (BMD) and bone volume relative to tissue volume (BV/TV, %) (E), and qRT-PCR analysis of ALP, Runx2, Osx, BSP and OCN (F). n = 5-7. (G and H) The body weight change of OVX female mice treated with or without GOLM1 treatment, treated with vehicle or combination of EGCG and BPTES (H). n=6-7.

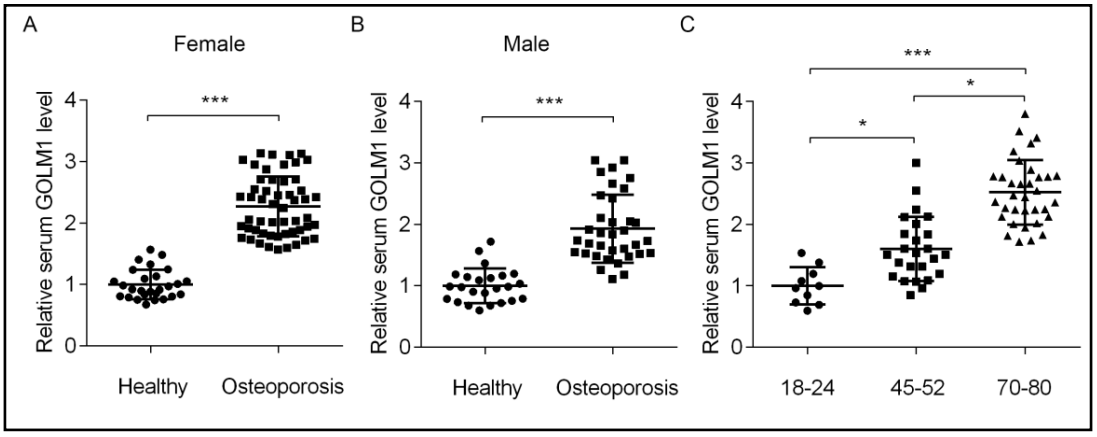

Fig. 6. Serum GOLM1 level increases in elder healthy people and osteoporosis patients. (A) Serum GOLM1 level was detected by Elisa kit in female osteoporosis (n=53) and age-matched healthy participants (n=27). (B) Serum GOLM1 level was detected by Elisa kit in male osteoporosis (n=35) and age-matched healthy participants (n=24). (C) Serum GOLM1 level was detected by Elisa kit in healthy participants of different age. n=10, 25 and 34.

GOLM1 or glutamine metabolism inhibition improves bone formation in osteoporosis

To investigate the role of GOLM1 or glutamine metabolism on osteoporosis *in vivo*, ovariectomy mouse model was used. Compared with the bones of Sham-operated mice, OVX-operated mouse BMSCs exhibited higher expression of GOLM1 (Fig. 5A). Glutamine uptake was increased in OVX-operated mouse BMSCs, accompanied with a reduction of intracellular glutamate and glutamine level (Fig. 5B). Furthermore, GOLM1 antibody significantly improved bone formation in OVX mouse model, as characterized by increased BMD and BV/TV (Fig. 5C). BMSCs isolated from the GOLM1 antibody-treated OVX mice showed higher expression of ALP, Runx2, Osx, BSP and OCN (Fig. 5D). Even though EGCG or BPTES alone had minimal effect on the BMD and BV/TV of OVX mice, their combination significantly enhanced the bone formation (Fig. 5E). Similarly, EGCG and BPTES combined treatment increased the expression of ALP, Runx2, Osx, BSP and OCN in BMSCs of OVX mice (Fig. 5F). The body weight change of OVX mice with ECGC and BPTES combined treatment was similar to the OVX mice without treatment, while GOLM1 antibody reduced the body weight, indicating that GOLM1 antibody might be toxic (Fig. 5G and 5H). In summary, our findings demonstrated that GOLM1 antibody or glutamate metabolism inhibitor could induce the osteogenic differentiation of BMSCs in mice, resulting in improved bone formation.

GOLM1 level is increased in the serum of elder people and osteoporosis patients

To explore the clinical relevance of our study, we checked the serum GOLM1 level in both female and male osteoporosis patients. GOLM1 abundance was increased in the serum of both female and male osteoporosis patients compared with that of healthy people (Fig. 6A and 6B). Since osteoporosis is an aging disease that tends to be more widely in elder patients, we also measured the serum GOLM1 level in different aged people. We found that GOLM1 was slightly increased in elder group compared with the younger group (Fig. 6C). Our results showed that GOLM1 might be a potential biomarker for osteoporosis.

Discussion

Osteoporosis is an aging disease which is characterized by microarchitectural deterioration of bone tissue and low bone mass. Although it is known that this disease is caused by an imbalance of bone homeostasis, the molecular mechanisms are largely

unknown. In this study, we identified GOLM1 as a potential biomarker for osteoporosis patients. The serum level of GOLM1 was increased in osteoporosis patients compared with age-matched healthy participants. Elder participants exhibited higher serum GOLM1 abundance than younger ones. These results suggested that GOLM1 might participate in the disease progression of osteoporosis.

Bone marrow mesenchymal stem cells (BMSCs) are progenitor cells of adipocytes, chondrocytes, and osteoblasts and play an important role in bone formation [6-8]. A reduced capacity of BMSCs differentiating into osteoblasts causes impaired bone formation, which contributes to osteoporosis. Osteogenic differentiation of BMSCs is regulated by various molecules and signaling pathways. Inflammation factors including Tumor necrosis factor α (TNF-α) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) have been reported to suppress BMSCs differentiating to osteoblasts [22, 23]. TNF-α directly inhibits the expression of Runx2, an osteoblast differentiation factor [24]. NFκB degradation of β-catenin leads to decreased differentiation of BMSCs to osteoblasts [13]. GOLM1 expression is up-regulated by virus infection, such as HBV, HCV and HIV [15, 16]. Since HBV or HCV infection is a major risk factor for chronic inflammation, GOLM1 which responses to HBV or HCV infection may be correlated with inflammation. However, the role of GOLM1 in BMSCs is unclear. In this study, we found that GOLM1 expression was down-regulated during the osteogenic differentiation of BMSCs, which is stimulated by Odi *in vitro*. By knocking down GOLM1, using GOLM1 antibody or over-expressing GOLM1, we showed that GOLM1 prevented the differentiation of BMSCs to osteoblast. Together with the clinical findings, our results suggested that GOLM1 may contribute to osteoporosis progression through inhibiting the osteogenic differentiation of BMSCs. However, since it is difficult to harvest the bone marrow from osteoporosis patients and healthy people, we could not illustrate whether the expression of GOLM1 changed in BMSCs of Human osteoporosis.

Glutamine metabolism has been shown to be important in regulating cell proliferation [25]. Glutamine was catalyzed to generate α-KG through two steps: glutamine to glutamate by glutaminase (GLS) and glutamate to α-KG by either glutamate dehydrogenase (GDH) or transaminases. Studies have reported that incorporation of α-KG into tricarboxylic acid (TCA) cycle promotes the production of nucleotides, lipids and amino acids, which are required for cell proliferation [26]. It is regulated by different signaling and dysregulation of glutamine metabolism has been found in various cancer development [27]. However, its role in BMSCs differentiation remains to be determined. Here, GDH activity and glutamine to α-KG conversion were reduced during osteogenic differentiation of BMSCs. By silencing GDH or using glutamine inhibitor EGCG, we found that inhibition of glutamine metabolism promoted the osteogenic differentiation of BMSCs. Previous studies have demonstrated that mTORC1 activation stimulates glutamate to α-KG conversion by repressing GDH activity, therefore promoting cancer cell proliferation [28]. c-Myc, an oncogenic transcription factor, activates glutamine metabolism through suppression of miR-23a/b, leading to accelerated tumor development [29]. Based on these studies, we found that GOLM1 over-expression activated mTOR signaling pathway. Rapamycin suppressed the glutamine metabolism in GOLM1 over-expressed BMSCs but had minimal effect in GOLM1 silencing BMSCs. In addition, GDH activity in GOLM1 over-expressed but not in GOLM1 knockdown BMSCs was suppressed by rapamycin treatment. Our results suggested that GOLM1 promoted glutamine metabolism depending on mTOR signaling pathway.

Although our findings illustrated an important role of GOLM1 in modulating BMSCs differentiation, our *in vitro* data could not conclude that GOLM1 serves as a pathological molecule in osteoporosis. Based on an ovariectomy mouse model, which is widely used to study osteoporosis, we found that GOLM1 antibody or combination of EGCG and BPTES could alleviate the osteoporosis in OVX-operated mice. These treatments induced the osteogenic differentiation of BMSCs. However, the body weight losing after GOLM1 antibody treatment suggested that it may not be safe when used for this disease. Our findings not only implied the critical role of GOLM1-mediated glutamine metabolism in BMSCs differentiation and osteoporosis, but also proposed a promising therapeutic target for this disease.

Conclusion

GOLM1 retarded the osteogenic differentiation of BMSCs. GOLM1 promoted glutamine metabolism through activation of mTOR signaling pathway. Inhibition of glutamine metabolism promoted the osteogenic differentiation of BMSCs. Importantly, GOLM1 antibody or inhibition of glutamate metabolism rescued the osteoporosis caused by ovariectomy operation. We also revealed the clinical relevance of GOLM1 in osteoporosis patients. Our research provides us a mechanistic insight into the BMSCs differentiation during osteoporosis and targeting the alterations may be a promising treatment for this disease.

Acknowledgements

This work was supported by Jiangsu Province's Young Medical Talents Program (QNRC2016880), National Natural Science Foundation of China Grant (81302438, 81572179) and Advantage discipline groups of the Second Affiliated Hospital of Soochow University (XKQ2015001).

Disclosure Statement

These authors declare no conflicts of interest.

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