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ORIGINAL RESEARCH

Genetic Evidence Supporting a Causal Association Between mTOR-Dependent EIF-4E Circulating Protein Level and Osteoporosis

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ABSTRACT

Introduction: The mechanistic target of rapamycin (mTOR) regulates bone homeostasis, a crucial factor in osteoporosis (OP) development. However, most research is based on observational studies, and the causality remains uncertain. Therefore, we analyzed two samples of mendelian randomization (MR) to determine whether there is a causal relationship between mTOR-dependent circulating proteins and OP.

Methods: Mendelian weighting (weighted median [WM], inverse variance weighting [IVW], and MR-Egger regression) were applied to analyze the causality between bone phenotypes (bone mineral density [BMD] in forearm, femoral neck, lumbar spine, and heel) and mTOR-dependent circulating proteins (RP-S6K, 4EBP, EIF-4E, EIF-4A, and EIF-4G). Horizontal pleiotropy and heterogeneities were detected using Cochran's Q test, MR-Pleiotropy RE-Sidual Sum and Outlier (MR-PRESSO), and "leave-one-out" analysis. The proteomics-GWAS INTERVAL study was used to select the instrumental variables (IVs) for mTOR proteins.

Results: As phenotypes for OP, estimations of BMD were taken in four different sites: forearm (FA) (n = 8143), femoral neck (FN) (n = 32,735),

Ting Cheng and Yao-Chen Zhang contributed equally to this work.

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lumbar spine (LS) (n = 28,498), and heel (eBMD) (n = 426,824). Based on IVW analysis, EIF4E is causally related to FA-BMD (OR = 0.938, 95% CI 0.887, 0.991, p = 0.024) but not to BMD elsewhere.

Conclusion: MR analysis revealed a causal relationship between EIF-4E and FA-BMD, which may provide new insights into the underlying pathogenesis of OP and a new therapeutic target for OP.

Keywords: Mendelian randomization; Osteoporosis; Mechanistic target of rapamycin; Bone mineral density; Eukaryotic translation initiation factor 4E

Key Summary Points

Why carry out this study?

Osteoporosis (OP) is a progressive, age-related, systemic disease featured by the loss of bone mass, leading to increased risk of fractures and reduced quality of life. The prevalence of OP is on the rise globally due to an aging population.

The mechanistic target-of-rapamycin (mTOR) regulates bone metabolism by promoting osteoblastic differentiation and increasing bone matrix synthesis. The association between mTOR and OP is largely based on observational studies, and causality remains uncertain.

This study conducted a mendelian randomization (MR) analysis to explore the causal relationship between mTOR-dependent proteins and bone mineral density (BMD), a characteristic index of OP.

What was learned from the study?

MR analysis revealed a causal association between mTOR-dependent EIF-4E circulating protein and forearm (FA)-BMD, providing new insights into the underlying pathophysiology of OP. Moreover, the study suggested that the PI3K/Akt/mTOR signaling pathway may be a therapeutic target for OP.

INTRODUCTION

Osteoporosis (OP) is a progressive, age-related, systemic disease characterized by the loss of bone mass and the deterioration of bone microstructure, leading to an increased risk of fractures [1, 2]. Globally, OP prevalence is on the rise due to an aging population [3, 4]. Bone mineral density (BMD) is a measurable and powerful index for diagnosing OP in the clinical field [5]. The WHO has established diagnostic criteria for osteoporosis by utilizing BMD T-scores.

Increasing evidence suggests that bone formation and resorption as well as osteoporosis treatment are affected by the mechanistic target-of-rapamycin (mTOR) pathway [6]. mTOR is a serine-threonine protein kinase that participates in the formation of two structural and functional complexes named mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [7]. The mTOR is critical cellular signaling that plays an essential role in regulating cell proliferation, autophagy, and apoptosis by coordinating the growth and metabolism of eukaryotic cells [7], which also serves as a regulator of bone metabolism related to promoting osteoblastic differentiation and increasing bone matrix synthesis [8, 9]. Xu et al. [10] reported that activation of mTORC1 in B lymphocytes promotes osteoclast formation. The mTORC1, which contains mTOR, G protein β-unit-like protein, and regulatory-associated protein of mTOR (Raptor) [11], regulates two downstream factors called ribosomal protein-S6 kinase (RP-S6K) and eukaryotic translation initiation factor 4E-binding protein (EIF4E-BP). The EIF4E-BP is a negative regulator of the EIF-4F complex, which comprises three proteins (EIF-4A, EIF-4E, and EIF-4G).

Although mounting evidence has emphasized the essential role of mTOR in regulating bone homeostasis, the causality remains uncertain since they are based on observational studies. Therefore, to explore the causal association between mTOR and OP, we conducted a mendelian randomization analysis of BMD at four sites: femur, forearm, lumbar spine, and calcaneus with five mTOR-dependent

circulating proteins: RP-S6K, 4EBP, EIF-4A, EIF-4E, and EIF-4G.

METHODS

Data Sources

In this study, BMD is regarded as a crucial index to evaluate the risk of osteoporosis. The following genome-wide association study (GWAS) data were obtained from a meta-analysis published at genetic factors for osteoporosis consortium (GEFOS) in 2015 [12]: forearm bone mineral density (FA-BMD), femoral neck bone mineral density (FN-BMD), and lumbar spine bone mineral density (LS-BMD). For FA-BMD, 8143 individuals were included. Meanwhile, a total of 32,735 individuals for FN-BMD and 28,489 individuals for FA-BMD were evaluated. All of the participants were from European populations. Based on sex, weight, age, and age squared, BMD was adjusted. Summary statistics for estimated heel bone mineral density (eBMD) were obtained from the IEU GWAS database [13], which contained 426,824 European participants, 55% of whom were female. We confirmed these data from the publicly available GWAS catalog website (https://www.ebi.ac.uk/gwas/downloads/summary-statistics) [14]. For the data about mTOR-dependent circulating proteins, a proteomics-GWAS INTERVAL study involving 3301 participants was conducted, which included 3600 plasma protein assays [15]. The study was performed in accordance with the Helsinki Declaration of 1964 and its later amendments. Patient consent for this study was not necessary because the study was non-interventional and data were de-identified.

Selection of Instrumental Variables

As instrumental variables (IVs), single-nucleotide polymorphisms (SNPs) related to mTOR-dependent circulating proteins were employed. These SNPs were strongly (p value 5×10^{-6}) and independently ($r^2 < 0.001$) linked with the exposure, satisfying the three assumptions for MR analysis which included the following: first, a genetic variation is associated with a particular exposure; second, confounding pathways are not affected by genetic variation in the outcomes; third, genetic variation will not affect the results directly but only indirectly through exposure. To reduce the possibility of statistical bias in the original GWSA, SNPs with a minor allele frequency (MAF) of < 0.01 were excluded because of their low confidence. SNPs with inconsistent alleles (e.g., T/C vs. T/G) and palindromic SNPs with ambiguous alleles (e.g., T/A vs. C/G) were harmonized by flipping the outcome variants. To exclude the instrumental SNPs showing a strong association with the outcomes and confounders, the PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/phenoscanner) was utilized. The exposure-related instrumental SNPs were then taken out of the outcome datasets. Finally, F-statistics was used to fulfill the relevance assumption, and F-statistics > 10 was regarded as valid instruments.

MR Analysis

MR analysis was used to estimate the causative effect of exposure variables on the outcome. In this study, we evaluated the causal associations between mTOR protein (RP-S6K, 4EBP, EIF-4A, EIF-4E, and EIF-4G) and BMD at different skeletal sites (FA-BMD, LS-BMD, FN-BMD, and eBMD) based on the summary statistics (β coefficients and standard errors). The MR analysis we used were inverse variance weighting (IVW), MR-Egger regression, and weighted median (WM). These MR methods were detailed in published studies [16, 17]. Among these methods, IVW is the most important for estimating the causal effect between exposure and outcome [18]. When there is no directional pleiotropy (p for MR-Egger intercept > 0.05) among the instrumental SNPs, the IVW method was considered the most reliable [19]. The MR analyses were performed with the R language and environment using the TwoSampleMR package [20]. We used the odds ratio (95% confidence interval) to express the estimated effect value, which can be recorded as per 1-SD

increment in each mTOR protein with the odds ratio (OR) of OP.

Sensitivity Analysis

Due to the absence of the assumption of pleiotropy in MR, the following sensitivity analysis was performed to verify the reliability of the analysis. First, Cochran's Q statistics were applied to assess the IVs' heterogeneity. Second, MR-Egger regression was used to determine whether the selected IVs had pleiotropic effects. Whether horizontal pleiotropy affects the results of MR analysis using the intercept term from MR-Egger regression was evaluated [21]. Third, in MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) analysis, a causal effect estimate was reduced by removing SNPs related to heterogeneity. Finally, we used leave-one-out validation to assess each IVs' sensitivity. Funnel plots were used to evaluate the heterogeneity among SNPs as well.

RESULTS

SNP Selection

As mentioned earlier, we selected independent SNPs ($r^2 < 0.001$, $p < 5 \times 10^{-6}$) associated with the exposure. Fifteen SNPs were extracted as significant predicted SNPs of RP-S6K. Fifteen SNPs were extracted as significant associated SNPs of 4EBP. Sixteen SNPs were selected as substantial predicted SNPs of EIF-4E. Nine SNPs were identified as significant predicted SNPs of EIF-4G. Ten SNPs were considered significant indicating SNPs of EIF-4A (Fig. 1). The mean F-statistic ranged from 28.02 to 52.69, which is above the standard cutoff (> 10) indicating sufficient instrumental strength. SNP-specific information is reported in Supplementary Tables. As a result, those significant SNPs of mTOR protein were eventually used as IVs for the MR analysis.

Causality Between mTOR Protein and OP

In the two-sample MR analysis, the level of EIF-4E had a strong causal relationship with FA-BMD (OR = 0.938, 95% CI 0.887, 0.991, $p = 0.024$) according to the IVW (Fig. 2A). However, RP-S6K, 4EBP, EIF-4A, and EIF-4G showed no causal relationship with FL-BMD, LS-BMD, FA-BMD, or eBMD in different MR methods ($p > 0.05$) (Fig. 2B–D, Table 1).

EIF-4E was causally associated with FA-BMD according to IVW analysis. Regarding sensitivity, no directional pleiotropy was evident in the MR-Egger finding (intercept = 0.010, $p = 0.59$). No single SNP strongly influenced causality between EIF-4E and FA-BMD in the leave-one-out analysis (Fig. 3). Based on the symmetry of the funnel plots, our study strictly adhered to the IV assumptions (Supplementary Figures). Moreover, there was no evidence of heterogeneity in IVW ($Q = 9.723$, $p = 0.716$). The remaining mTOR-dependent circulating proteins are shown in the Supplementary Figures.

DISCUSSION

In the present study, we identified causal associations of mTOR-dependent circulating proteins with various skeletal sites. The results indicated that a genetically higher level of EIF-4E was positively associated with higher FA-BMD in the IVW analysis. In addition, we found no association between proteins (RP-S6K, 4EBP, EIF-4A, and EIF-4G) with FN-BMD, LS-BMD, and eBMD.

As the elderly population grows, OP gradually becomes a global health problem. The imbalance between bone resorption and formation results in a number of disorders including osteoporosis [22]. Bone resorption and osteoclast differentiation are regulated by autophagy-related biological processes [23]. Autophagy inhibition reduces the differentiation of osteoclast progenitors into osteoclasts and causes osteoclast dysfunction [24]. In addition, osteoblasts are formed by mesenchymal stem cells (MSCs) that undergo autophagy during bone formation [25]. Inhibition of osteocyte autophagy leads to aging-related OP

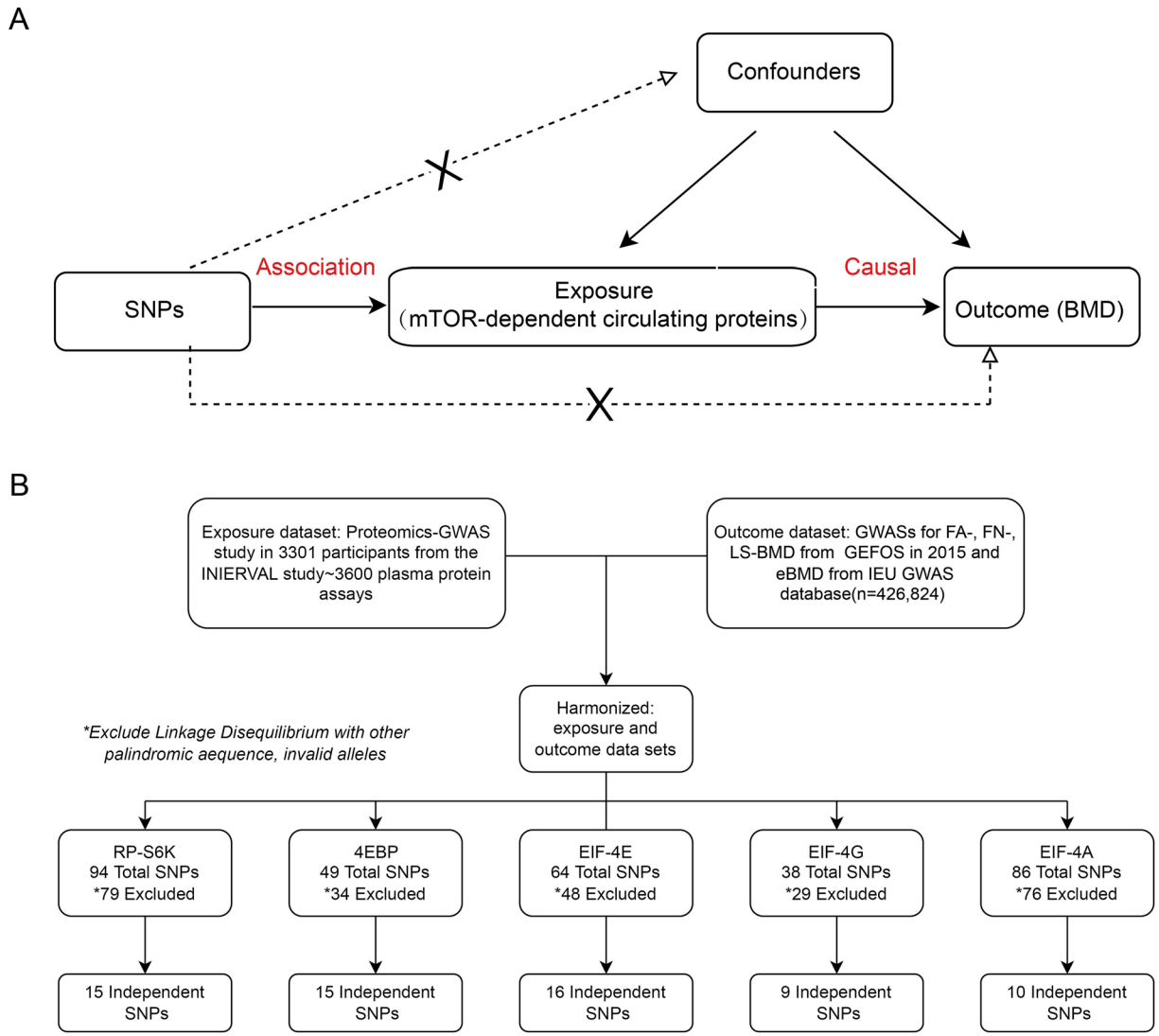
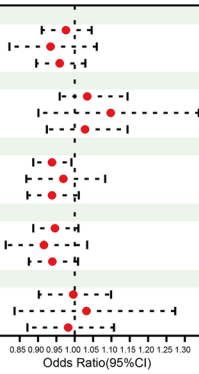


Fig. 1 Schematic representation of MR. **A** Casual-directed acyclic graph. Relationships between variables are indicated by directed arrows, suggesting a direct causal effect, and dashed arrows, showing no direct effect. **B** Design flow chart. *SNP* single-nucleotide polymorphism, *GWAS* genome-wide association study, *GEFOS* genetic factors for osteoporosis consortium, *BMD* bone mineral density, *FN* femoral neck, *LS* lumbar spine, *FA* forearm, *RP-S6K* ribosomal protein-S6 kinase, *4EBP* 4E-binding protein, *EIF-4E* eukaryotic translation initiation factor 4E, *EIF-4A* eukaryotic translation initiation factor 4A, *EIF-4G* eukaryotic translation initiation factor 4G

[26]. The mTOR pathway is a crucial repressor of autophagy. Upstream signaling pathways including PI3K/AKT/mTOR mediate the inhibition of autophagy induced by mTOR (Fig. 4). As shown in Fig. 4, PI3K is activated by insulin, glucose, and cytokines, which phosphorylate PDK1 at Thr308, triggering protein kinase B (AKT) phosphorylation [27]. Alternatively, mTORC2 can also mediate the activation of AKT for its phosphorylation at Ser473 [28]. Activated AKT transfers to other cell compartments to activate various downstream substrates, and mTORC1 is a critical downstream branch [29]. Meanwhile, as a result of phosphorylating AKT, mTORC1 is directly activated at Ser 2448 by phosphorylated mTOR [29].

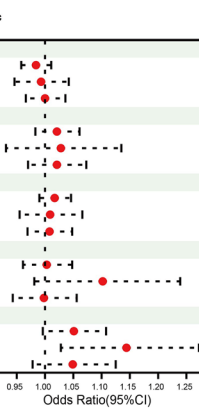
A

Exposure	Outcome	N of SNPs	Cochran's Q statistic (p-value)	OR(95%CI)	p-value	intercept
RP-S6K	FA-BMD					
Inverse variance weighted		13	20.243(0.063)	0.976(0.910, 1.046)	0.488	
MR Egger		13	19.086(0.059)	0.934(0.822, 1.060)	0.313	0.0151093
Weighted median		13		0.959(0.895, 1.029)	0.246	
4EBP-1	FA-BMD					
Inverse variance weighted		12	9.883(0.541)	1.034(0.959, 1.144)	0.384	
MR Egger		12	9.465(0.488)	1.098(0.901, 1.338)	0.375	-0.01187147
Weighted median		12		1.028(0.924, 1.144)	0.611	
EIF-4E	FA-BMD					
Inverse variance weighted		14	9.723(0.716)	0.938(0.887, 0.991)	**0.024**	
MR Egger		14	9.260(0.680)	0.969(0.868, 1.083)	0.596	-0.01036101
Weighted median		14		0.938(0.871, 1.011)	0.095	
EIF-4A	FA-BMD					
Inverse variance weighted		10	10.313(0.325)	0.946(0.877, 1.010)	0.097	
MR Egger		10	9.823(0.278)	0.916(0.812, 1.034)	0.195	0.008151014
Weighted median		10		0.939(0.875, 1.009)	0.089	
EIF-4G	FA-BMD					
Inverse variance weighted		7	3.449(0.750)	0.996(0.902, 1.009)	0.935	
MR Egger		7	3.304(0.653)	1.032(0.836, 1.274)	0.777	-0.007571771
Weighted median		7		0.982(0.871, 1.107)	0.765	



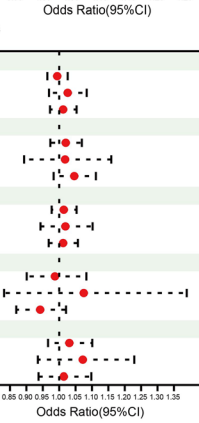
B

Exposure	Outcome	N of SNPs	Cochran's Q statistic (p-value)	OR(95%CI)	p-value	intercept
RP-S6K	FN-BMD					
Inverse variance weighted		13	8.458(0.748)	0.984(0.958,1.011)	0.256	
MR Egger		13	8.273(0.689)	0.993(0.946,1.042)	0.788	-0.002948
Weighted median		13		1.000(0.966,1.036)	0.965	
4EBP-1	FN-BMD					
Inverse variance weighted		11	9.080(0.524)	1.021(0.983,1.061)	0.265	
MR Egger		11	9.062(0.413)	1.028(0.931,1.135)	0.595	-0.001287
Weighted median		11		1.021(0.970,1.073)	0.429	
EIF-4E	FN-BMD					
Inverse variance weighted		14	10.857(0.623)	1.017(0.990,1.046)	0.206	
MR Egger		14	10.733(0.552)	1.009(0.955,1.066)	0.747	0.002624
Weighted median		14		1.008(0.969,1.048)	0.680	
EIF-4A	FN-BMD					
Inverse variance weighted		9	6.362(0.607)	1.003(0.961,1.048)	0.861	
MR Egger		9	3.494(0.836)	1.102(0.981,1.239)	0.146	-0.014972
Weighted median		9		0.998(0.943,1.056)	0.948	
EIF-4G	FN-BMD					
Inverse variance weighted		6	3.605(0.607)	1.051(0.996,1.108)	0.068	
MR Egger		6	0.352(0.986)	1.144(1.028,1.272)	0.068	-0.018214
Weighted median		6		1.049(0.978,1.125)	0.0176	



C

Exposure	Outcome	N of SNPs	Cochran's Q statistic (p-value)	OR(95%CI)	p-value	intercept
RP-S6K	LS-BMD					
Inverse variance weighted		13	9.613(0.649)	0.994(0.964, 1.026)	0.731	
MR Egger		13	7.936(0.718)	1.026(0.969, 1.084)	0.395	-0.010347
Weighted median		13		1.012(0.972, 1.053)	0.565	
4EBP-1	LS-BMD					
Inverse variance weighted		11	11.541(0.317)	1.020(0.973, 1.069)	0.405	
MR Egger		11	11.539(0.241)	1.018(0.893, 1.159)	0.792	0.012702
Weighted median		11		1.046(0.983, 1.112)	0.151	
EIF-4E	LS-BMD					
Inverse variance weighted		14	17.793(0.165)	1.014(0.977, 1.053)	0.447	
MR Egger		14	17.766(0.123)	1.019(0.943, 1.102)	0.638	-0.001411
Weighted median		14		1.012(0.968, 1.057)	0.587	
EIF-4A	LS-BMD					
Inverse variance weighted		9	26.805(0.001)	0.987(0.901, 1.083)	0.786	
MR Egger		9	25.031(0.001)	1.075(0.833, 1.389)	0.595	-0.013679
Weighted median		9		0.942(0.869, 1.021)	0.142	
EIF-4G	LS-BMD					
Inverse variance weighted		6	5.627(0.344)	1.013(0.966, 1.101)	0.354	
MR Egger		6	5.096(0.277)	1.072(0.935, 1.229)	0.375	-0.008476
Weighted median		6		1.014(0.937, 1.098)	0.721	



D

Exposure	Outcome	N of SNPs	Cochran's Q statistic (p-value)	OR(95%CI)	p-value	intercept
RP-S6K	eBMD					
Inverse variance weighted		13	53.327($3.6e^{-7}$)	1.001(0.988,1.015)	0.809	
MR Egger		13	45.397($4.13e^{-6}$)	1.015(0.992,1.038)	0.222	-0.004683
Weighted median		13		0.999(0.991,1.007)	0.835	
4EBP-1	eBMD					
Inverse variance weighted		11	49.888($2.79e^{-7}$)	1.009(0.989,1.031)	0.366	
MR Egger		11	45.248($8.29e^{-7}$)	1.034(0.981,1.091)	0.248	-0.004662
Weighted median		11		1.013(0.997,1.029)	0.109	
EIF-4E	eBMD					
Inverse variance weighted		13	33.108(0.001)	1.006(0.996,1.017)	0.239	
MR Egger		13	31.491(0.001)	0.999(0.977,1.021)	0.638	0.003055
Weighted median		13		1.002(0.993,1.011)	0.571	
EIF-4A	eBMD					
Inverse variance weighted		10	30.589(0.001)	0.999(0.986,1.012)	0.963	
MR Egger		10	29.279(0.001)	0.993(0.969,1.018)	0.616	0.001575
Weighted median		10		1.001(0.991,1.009)	0.885	
EIF-4G	eBMD					
Inverse variance weighted		8	74.031($2.25e^{-13}$)	0.971(0.939,1.004)	0.089	
MR Egger		8	60.469($3.61e^{-11}$)	1.007(0.939,1.081)	0.844	-0.007977
Weighted median		8		0.999(0.982,1.107)	0.997	

◀**Fig. 2** Risk of mTOR protein for genetically predicted osteoporosis. MR-Egger intercept evaluates the possibility of pleiotropy. **A** FA-BMD. **B** FN-BMD. **C** LS-BMD. **D** eBMD. *OR* odds ratio, *CI* confidence interval, *MR-Egger* mendelian randomization-Egger regression, *FN-BMD* femoral neck-bone mineral density, *LS-BMD* lumbar spine-bone mineral density, *FA-BMD* forearm-bone mineral density, *eBMD* heel bone mineral density, *RP-S6K* ribosomal protein-S6 kinase, *4EBP* 4E-binding protein, *EIF-4E* eukaryotic translation initiation factor 4E, *EIF-4A* eukaryotic translation initiation factor 4A, *EIF-4G* eukaryotic translation initiation factor 4G

MTORC1 is a modulator of ULK1 and is associated with autophagy. Specifically, mTORC1 inhibits autophagy by phosphorylating ULK1 at serine 757 (ULK1-PS757) [30]. Therefore, mTOR may have been linked to the pathogenesis of OP.

A previous clinical observation found that rapamycin, an mTOR inhibitor, could augment regulatory T cells (Tregs) [31], which were markedly reduced in patients with OP [32]. The immune effect of rapamycin may be associated with OP because of its regulation of Treg cells. The osteoclasts are key mediators of skeletal diseases, especially osteoporosis. The receptor activators of the nuclear factor-κb ligand (RANKL)/RANK/osteoprotegerin (OPG) signaling system is the major regulator of osteoclast biology. This signaling pathway is made up of a triad of RANKL RANK, and OPG, which are all members of the tumor necrosis factor (TNF-) superfamily [33]. According to Choi and Arron [34] in 2000, bone resorption and T cells are related, and "bone immunology" is further defined as the study of the relationship between bone resorption and T cells. The Th17 cells promote bone resorption in both direct and indirect ways. On the one hand, osteoclast precursors express RANKL [35], which leads to osteoclastic differentiation by binding to RANK [36]. Furthermore, the Th17 cytokines strongly induced RANKL expression [34]. On the other hand, IL-17 secreted by Th17 cells promotes RANKL expression in osteoclastogenesis-supporting cells, increasing bone resorption [37]. Treg cells can inhibit osteoclast formation by preventing RANKL expression [38]. It has been demonstrated that Treg cells impact bone mainly through a contact-dependent mechanism and an inhibitory cytokine-dependent mechanism [39]. In addition, either activation of intracellular effectors by Treg cells or TGF-β secreted by Treg can promote osteoblast proliferation and differentiation [40].

Table 1 MR estimates the causality between mTOR protein and osteoporosis

Sample	FN-BMD	LS-BMD	FA-BMD	eBMD
RP-S6K	0.256	0.731	0.488	0.809
4EBP	0.264	0.406	0.384	0.366
EIF-4E	0.206	0.447	**0.024**	0.238
EIF-4A	0.861	0.786	0.097	0.963
EIF-4G	0.068	0.354	0.935	0.089

FN-BMD femoral neck-bone mineral density, *LS-BMD* lumbar spine-bone mineral density, *FA-BMD* forearm-bone mineral density, *eBMD* heel bone mineral density, *RP-S6K* ribosomal protein-S6 kinase, *4EBP* 4E-binding protein, *EIF-4E* eukaryotic translation initiation factor 4E, *EIF-4A* eukaryotic translation initiation factor 4A, *EIF-4G* eukaryotic translation initiation factor 4G

We found a causal relationship between EIF-4E and FA-BMD, suggesting the mTOR pathway is a possible therapeutic target for OP. The EIF-4E is a downstream target of mTORC1/4EBP. The mTORC1 controls the translation process through the phosphorylation of EIF4EBP. Unphosphorylated 4EBP binds to EIF-4E and inhibits the formation of translation initiation complex 4F (eIF4F) to prevent translation [41] (Fig. 4). Takashi Iezaki et al. [42] reported that the mTORC1/4EBP cascade regulates the translation of NRA in undifferentiated mesenchymal cells, indicating the role of the mTORC1/4EBP axis in skeletal development. Currently, EIF-4E has been involved in tumorigenesis. It has been considered a potential therapeutic target in bone cancer [43, 44], but the study of EIF-4E in OP is still in its infancy. Results from our MR analyses suggested that the causal association between EIF-4E and FA-BMD may be related to the following factors shown in Fig. 4: first, EIF-4E serves an essential role in the PI3K/AKT/

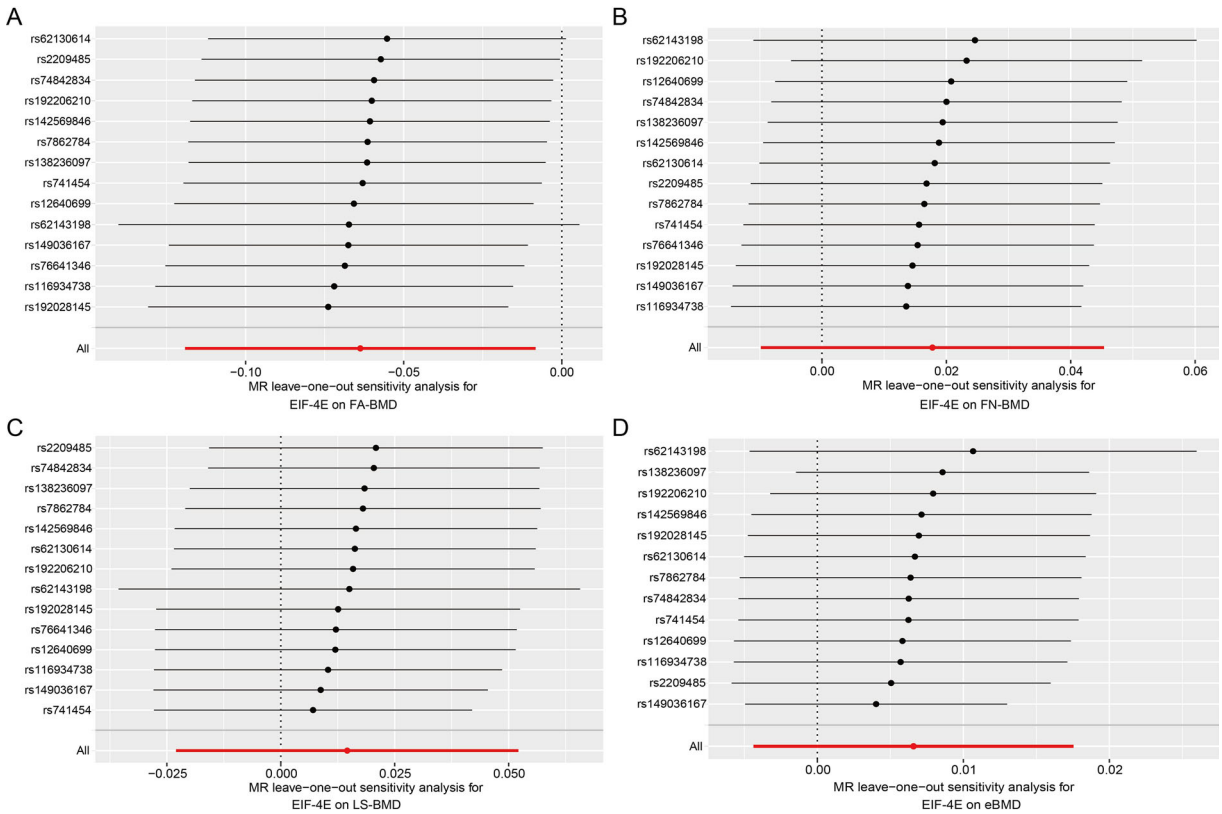


Fig. 3 MR leave-one-out sensitivity analysis for EIF-4E on osteoporosis. **A** FA-BMD. **B** FN-BMD. **C** LS-BMD. **D** eBMD. Dots indicate MR analysis of osteoporosis using inverse variance-weighted fixed effects if each SNP is omitted entirely. The bars indicate the CI. *FN-BMD* femoral neck-bone mineral density, *LS-BMD* lumbar spine-bone mineral density, *FA-BMD* forearm-bone mineral density, *eBMD* heel bone mineral density, *RP-S6K* ribosomal protein-S6 kinase, *4EBP* 4E-binding protein, *EIF-4E* eukaryotic translation initiation factor 4E, *EIF-4A* eukaryotic translation initiation factor 4A, *EIF-4G* eukaryotic translation initiation factor 4G

mTOR pathway, regulating protein and lipid metabolism, which affects the balance between bone formation and resorption. Second, the mTORC1 inhibition downregulated the synthesis of Tregs by downregulated EIF-4E-dependent mRNA translation, thus destroying the homeostasis of the immune system [45]. Also, a deficiency of Tregs was found in patients with OP [46]. Prospective studies are required to confirm the causal relationship between EIF-4E and OP.

Although MR can strengthen causal inference, it also has limitations. Since population stratification affects the genotype-disease relationship, our regression model adjusted for race. Notably, it did not influence the strength of the correlation. However, given that all the data we used were from European ancestry, the results can only be generalized to essentially healthy European adults. Second, two-sample MR analysis requires non-overlapping samples between exposure and outcome GWAS, but we could not accurately estimate the overlap of the sampled copies. However, it is possible to minimize the bias associated with sample overlap using strong instruments.

CONCLUSION

This study used metabolomics to explore the relationship between osteoporosis and mTOR proteins. MR analysis revealed a causal association between EIF-4E and FA-BMD. Considering

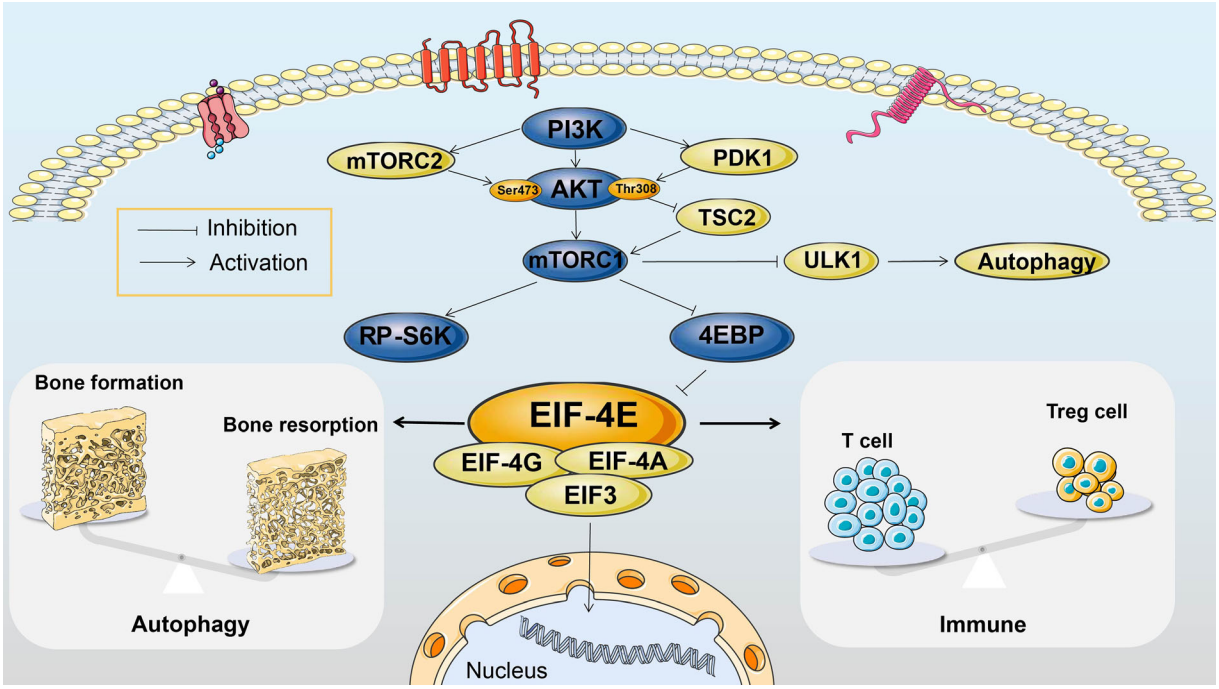


Fig. 4 Simplified model showing PI3K/AKT/mTOR signaling. This scheme offers the regulatory role of EIF-4E in autophagy and immunity. *PI3K* phosphatidylinositol 3-kinase, *AKT* protein; kinase B, *mTOR* mechanistic target of rapamycin, *RP-S6K* ribosomal protein-S6 kinase, *4EBP* 4E-binding protein, *EIF-4E* eukaryotic translation initiation factor 4E, *EIF-4A* eukaryotic translation initiation factor 4A, *EIF-4G* eukaryotic translation initiation factor 4G

BMD is the gold standard test for diagnosing OP, the discoveries shed light on the underlying pathophysiological mechanisms contributing to the development of OP. Moreover, the study suggested that the PI3K/Akt/mTOR signaling pathway may be a therapeutic target for OP.

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Data Availability. These data were derived from the following resources available in the public websites: the exposure data for mTOR-dependent circulating proteins (https://www.phpc.cam.ac.uk/ceu/proteins/), and the

outcome data for BMD (https://gwas.mrcieu.ac.uk/datasets/).

Declarations

Conflict of Interest. Ting Cheng, Yao-Chen Zhang, Ke-Yi Fan, Jing-Xi Hu, Qian Wang, Qi Wang, Liu Liu, He-Yi Zhang, Yao-Pu Hou, Xiao-Feng Li, Sheng-Xiao Zhang have no conflicts of interest to disclose.

Ethical Approval. The study was performed in accordance with the Helsinki Declaration of 1964 and its later amendments. Patient consent for this study was not necessary because the study was non-interventional and data were de-identified.

REFERENCES

1. Compston JE, McClung MR, Leslie WD. Osteoporosis. Lancet (London, England). 2019;393(10169):364–76.
2. Ensrud KE, Crandall CJ. Osteoporosis. Ann Intern Med. 2017;167(3):17–32.
3. Holroyd C, Cooper C, Dennison E. Epidemiology of osteoporosis. Best Pract Res Clin Endocrinol Metab. 2008;22(5):671–85.
4. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. J Bone Miner Res. 2007;22(3):465–75.
5. Kanis JA, Borgstrom F, De Laet C, Johansson H, Johnell O, Jonsson B, Oden A, Zethraeus N, Pfleger B, Khaltaev N. Assessment of fracture risk. Osteoporos Int. 2005;16(6):581–9.
6. Jeyabalan J, Shah M, Viollet B, Chenu C. AMP-activated protein kinase pathway and bone metabolism. J Endocrinol. 2012;212(3):277–90.
7. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. Cell. 2017;168(6):960–76.
8. Liu DM, Zhao L, Liu TT, Jiao PL, Zhao DD, Shih MS, Tao B, Sun LH, Zhao HY, Liu JM. Rictor/mTORC2 loss in osteoblasts impairs bone mass and strength. Bone. 2016;90:50–8.
9. Zhan JK, Wang YJ, Wang Y, Wang S, Tan P, Huang W, Liu YS. The mammalian target of rapamycin signalling pathway is involved in osteoblastic differentiation of vascular smooth muscle cells. Can J Cardiol. 2014;30(5):568–75.
10. Roman B, Montei C, Zhang L, Biswas P, Donofrio R, Xu S, Zhang Y, Liu B, Li K, Huang B, Yan B, Zhang Z, Liang K, Jia C, Lin J, Zeng C, Cai D, Jin D, Jiang Y, Bai X. Activation of mTORC1 in B lymphocytes promotes osteoclast formation via regulation of β-catenin and RANKL/OPG. J AOAC Int. 2016;31(7):1320–33.
11. Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol. 2011;12(1):21–35.
12. Zheng HF, Forgetta V, Hsu YH, Estrada K, Rosello-Diez A, Leo PJ, Dahia CL, Park-Min KH, Tobias JH, Kooperberg C, Kleinman A, Styrkarsdottir U, Liu CT, Uggla C, Evans DS, Nielson CM, Walter K, Pettersson-Kymmer U, McCarthy S, Eriksson J, Kwan T, Jhamai M, Trajanoska K, Memari Y, Min J, Huang J, Danecek P, Wilmot B, Li R, Chou WC, Mokry LE, Moayyeri A, Claussnitzer M, Cheng CH, Cheung W, Medina-Gómez C, Ge B, Chen SH, Choi K, Oei L, Fraser J, Kraaij R, Hibbs MA, Gregson CL, Paquette D, Hofman A, Wibom C, Tranah GJ, Marshall M, Gardiner BB, Cremin K, Auer P, Hsu L, Ring S, Tung JY, Thorleifsson G, Enneman AW, van Schoor NM, de Groot LC, van der Velde N, Melin B, Kemp JP, Christiansen C, Sayers A, Zhou Y, Calderari S, van Rooij J, Carlson C, Peters U, Berlivet S, Dostie J, Uitterlinden AG, Williams SR, Farber C, Grinberg D, LaCroix AZ, Haessler J, Chasman DI, Giulianini F, Rose LM, Ridker PM, Eisman JA, Nguyen TV, Center JR, Nogues X, Garcia-Giralt N, Launer LL, Gudnason V, Mellström D, Vandenput L, Amin N, van Duijn CM, Karlsson MK, Ljunggren Ö, Svensson O, Hallmans G, Rousseau F, Giroux S, Bussière J, Arp PP, Koromani F, Prince RL, Lewis JR, Langdahl BL, Hermann AP, Jensen JE, Kaptoge S, Khaw KT, Reeve J, Formosa MM, Xuereb-Anastasi A, Åkesson K, McGuigan FE, Garg G, Olmos JM, Zarrabeitia MT, Riancho JA, Ralston SH, Alonso N, Jiang X, Goltzman D, Pastinen T, Grundberg E, Gauguier D, Orwoll ES, Karasik D, Davey-Smith G, Smith AV, Siggeirsdottir K, Harris TB, Zillikens MC, van Meurs JB, Thorsteinsdottir U, Maurano MT, Timpson NJ, Soranzo N, Durbin R, Wilson SG, Ntzani EE, Brown MA, Stefansson K, Hinds DA, Spector T, Cupples LA, Ohlsson C, Greenwood CM, Jackson RD, Rowe DW, Loomis CA, Evans DM, Ackert-Bicknell CL, Joyner AL, Duncan EL, Kiel DP, Rivadeneira F, Richards JB. Whole-genome sequencing identifies EN1 as a determinant of bone density and fracture. Nature. 2015;526(7571):112–7.

13. Morris JA, Kemp JP. An atlas of genetic influences on osteoporosis in humans and mice. Nat Genet. 2019;51(2):258–66.

14. Tang Y. Absence of causal association between Vitamin D and bone mineral density across the lifespan: a Mendelian randomization study. Nat Genet. 2022;12(1):10408.

15. Sun BB, Maranville JC, Peters JE, Stacey D, Staley JR, Blackshaw J, Burgess S, Jiang T, Paige E, Surendran P, Oliver-Williams C, Kamat MA, Prins BP, Wilcox SK, Zimmerman ES, Chi A, Bansal N, Spain SL, Wood AM, Morrell NW, Bradley JR, Janjic N, Roberts DJ, Ouwehand WH, Todd JA, Soranzo N, Suhre K, Paul DS, Fox CS, Plenge RM, Danesh J, Runz H, Butterworth AS. Genomic atlas of the human plasma proteome. Sci Rep. 2018;558(7708): 73–9.

16. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet. 2014;23(R1): R89-98.

17. Didelez V, Sheehan N. Mendelian randomization as an instrumental variable approach to causal inference. Stat Methods Med Res. 2007;16(4):309–30.

18. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol. 2013;37(7):658–65.

19. Holmes MV, Ala-Korpela M, Smith GD. Mendelian randomization in cardiometabolic disease: challenges in evaluating causality. Nat Rev Cardiol. 2017;14(10):577–90.

20. Hemani G, Zheng J. The MR-Base platform supports systematic causal inference across the human phenome. Elife. 2018;7: e34408.

21. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. Eur J Epidemiol. 2017;32(5):377–89.

22. Hocking LJ, Whitehouse C, Helfrich MH. Autophagy: a new player in skeletal maintenance? J Immunol Res. 2012;27(7):1439–47.

23. Zhang L, Guo YF, Liu YZ, Liu YJ, Xiong DH, Liu XG, Wang L, Yang TL, Lei SF, Guo Y, Yan H, Pei YF, Zhang F, Papasian CJ, Recker RR, Deng HW. Pathway-based genome-wide association analysis identified the importance of regulation-of-autophagy pathway for ultradistal radius BMD. J Bone Miner Res. 2010;25(7):1572–80.

24. Sugatani T, Hruska KA. Akt1/Akt2 and mammalian target of rapamycin/Bim play critical roles in osteoclast differentiation and survival, respectively, whereas Akt is dispensable for cell survival in isolated osteoclast precursors. J Biol Chem. 2005;280(5):3583–9.

25. Darcy A, Meltzer M, Miller J, Lee S, Chappell S, Ver Donck K, Montano M. A novel library screen identifies immunosuppressors that promote osteoblast differentiation. Bone. 2012;50(6):1294–303.

26. Manolagas SC, Parfitt AM. What old means to bone. Trends Endocrinol Metab. 2010;21(6):369–74.

27. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. Curr Biol CB. 1997;7(4):261–9.

28. Huang J, Manning BD. A complex interplay between Akt, TSC2 and the two mTOR complexes. Biochem Soc Trans. 2009;37(Pt 1):217–22.

29. Manning BD, Toker A. AKT/PKB signaling: navigating the network. Cell. 2017;169(3):381–405.

30. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol. 2011;13(2): 132–41.

31. Wen HY, Wang J. Low-dose sirolimus immunoregulation therapy in patients with active rheumatoid arthritis: a 24-week follow-up of the randomized, open-label, parallel-controlled trial. J Immunol Res. 2019;2019:7684352.

32. Beck N. Validation of modifications to the Soleris® *E. coli* method for detection and threshold determination of *Escherichia coli* in select foods: level 3 modification to AOAC performance tested method SM 101101. Nat Commun. 2022;105(2):483–91.

33. Tobeiha M, Moghadasian MH, Amin N, Jafarnejad S. RANKL/RANK/OPG pathway: a mechanism involved in exercise-induced bone remodeling. J Immunol Res. 2020;2020:6910312.

34. Takayanagi H. Osteoimmunology and the effects of the immune system on bone. Nat Rev Rheumatol. 2009;5(12):667–76.

35. Hsu H, Lacey DL, Dunstan CR, Solovyev I, Colombero A, Timms E, Tan HL, Elliott G, Kelley MJ, Sarosi I, Wang L, Xia XZ, Elliott R, Chiu L, Black T, Scully S, Capparelli C, Morony S, Shimamoto G, Bass MB, Boyle WJ. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Biomed Res Int. 1999;96(7):3540–5.

36. Takayanagi H, Sato K, Takaoka A, Taniguchi T. Interplay between interferon and other cytokine

systems in bone metabolism. Immunol Rev. 2005;208:181–93.

37. Ono T, Takayanagi H. Osteoimmunology in bone fracture healing. Curr Osteoporos Rep. 2017;15(4): 367–75.

38. Okamoto K, Nakashima T, Shinohara M, Negishi-Koga T, Komatsu N, Terashima A, Sawa S, Nitta T, Takayanagi H. Osteoimmunology: the conceptual framework unifying the immune and skeletal systems. Physiol Rev. 2017;97(4):1295–349.

39. Zaiss MM, Axmann R, Zwerina J, Polzer K, Gückel E, Skapenko A, Schulze-Koops H, Horwood N, Cope A, Schett G. Treg cells suppress osteoclast formation: a new link between the immune system and bone. Arthritis Rheum. 2007;56(12):4104–12.

40. Runyan CE, Liu Z, Schnaper HW. Phosphatidylinositol 3-kinase and Rab5 GTPase inversely regulate the Smad anchor for receptor activation (SARA) protein independently of transforming growth factor-β1. J Biol Chem. 2012;287(43):35815–24.

41. Blagden SP, Willis AE. The biological and therapeutic relevance of mRNA translation in cancer. Nat Rev Clin Oncol. 2011;8(5):280–91.

42. Iezaki T, Horie T, Fukasawa K, Kitabatake M, Nakamura Y, Park G, Onishi Y, Ozaki K, Kanayama T, Hiraiwa M, Kitaguchi Y, Kaneda K, Manabe T, Ishigaki Y, Ohno M, Hinoi E. Translational control of Sox9 RNA by mTORC1 contributes to skeletogenesis. Stem Cell Rep. 2018;11(1):228–41.

43. Li S, Fu J, Lu C, Mapara MY, Raza S, Hengst U, Lentzsch S. Elevated translation initiation factor eIF4E is an attractive therapeutic target in multiple myeloma. J Exp Clin Cancer Res CR. 2016;15(4): 711–9.

44. Zuo D, Shogren KL, Zang J, Jewison DE, Waletzki BE, Miller AL 2nd, Okuno SH, Cai Z, Yaszemski MJ, Maran A. Inhibition of STAT3 blocks protein synthesis and tumor metastasis in osteosarcoma cells. J Exp Clin Cancer Res. 2018;37(1):244.

45. Volta V, Pérez-Baos S. A DAP5/eIF3d alternate mRNA translation mechanism promotes differentiation and immune suppression by human regulatory T cells. Nat Commun. 2021;12(1):6979.

46. Cheng T, Zhang SX, Wang J, Qiao J, Chang MJ, Niu HQ, Liu GY, Li XF. Abnormalities of peripheral lymphocyte subsets in rheumatoid arthritis patients complicated with osteoporosis. Rheumatol Ther. 2022;9(4):1049–59.