Mechano growth factor E peptide regulates migration and differentiation of bone marrow mesenchymal stem cells

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Abstract
IGF1Ec in humans or IGF1Eb in rodents (known as mechano growth factor (MGF)) has a unique E domain, and the C-terminal end of the E domain (MGF E peptide) plays important roles in proliferation, migration and differentiation of many cell types. Bone marrow mesenchymal stem cells (BMSCs) have multiple differentiation potentials and are considered as perfect seed cells for tissue repair. But the role of MGF E peptide on BMSCs is seldom investigated and the mechanism is still unclear. In this study, we investigated the effects of MGF E peptide on rat BMSCs (rBMSCs). Our results revealed that treatment with MGF E peptide had no effect on BMSC proliferation. However, both wound-healing and transwell assays indicated that MGF E peptide could significantly enhance rBMSCs migration ability. Further analysis indicated that MGF E peptide also reduced the expression levels of osteogenic genes, but increased the expression levels of adipogenic genes. Analysis of molecular mechanism showed that phosphorylation-Erk1/2 was activated by MGF E peptide and blockage of either Erk1/2 or IGF1 receptor could repress the migration effect of MGF E peptide. In conclusion, MGF E peptide is able to inhibit osteogenic differentiation but promote adipogenic differentiation. In addition, the migration effect of MGF E peptide on rBMSCs depends on IGF1 receptor via Erk1/2 signal pathway.

Key Words
- MGF E peptide
- mesenchymal stem cells
- migration
- differentiation
- Erk1/2 signal pathway
- IGF1 receptor

Introduction
Insulin-like growth factor 1 (IGF1) is a crucial systemic factor produced by the liver and is involved in the regulation of growth in many tissues (Liu et al. 1993). Many reports have revealed that IGF1 has a wide vary of functions, such as promoting cell proliferation, migration and inhibition of apoptosis (Li et al. 2007, Vanamala et al. 2010, Brisson & Barton 2012). In humans, the pre-mRNA of IGF1 can generate three isoforms, IGF1EA, IGF1EB and IGF1Ec, by alternative splicing (in rodents two isoforms: IGF1EA and IGF1EB). These three isoforms contain the same mature IGF1 peptide but in a different E domain. The different E domains of IGF1 contain isoforms that have special functions (Dai et al. 2010).

IGF1Ec in human (IGF1Eb in rodents), which is also named as mechano growth factor (MGF), was first identified by Goldspink (McKoy et al. 1999, Yang et al. 1996) in the
muscle cells in response to mechanical stimuli. Further research revealed that MGF was activated in heart, brain and bone by ischaemia, local damage and before stimuli respectively (Dluzniewska et al. 2005, Tang et al. 2006, Stavropoulou et al. 2009). A previous study found that a 49 bp insert (a 52 bp insert in rodents) during the splicing of exons 5 gives rise to a unique E domain of MGF (MGF E peptide) (Dai et al. 2010). It is reported that MGF E peptide activated myoblasts C2C12 and myocardial cells H9C2 proliferation, while delaying the differentiation of C2C12 (Yang & Goldspink 2002, Kandalla et al. 2011). In addition, in vivo and in vitro experiments indicated that MGF E peptide displayed significant neuroprotective effects in brain ischaemia (Dluzniewska et al. 2005). In conclusion, a series of evidence suggested that MGF E peptide serve as a separate growth factor and showed different functions for IGF1Ra.

Bone marrow mesenchymal stem cells (BMSCs) have multiple differentiation potentials with self-renewal capabilities and can be differentiated into many tissue-specific lineages, including osteoblasts, chondroblasts, adipocytes and others (Pittenger et al. 1999). BMSCs are potential perfect seed cells for tissue repair, as they display unique features, such as immunoregulatory and wide source abilities and can be differentiated into many tissue-specific lineages, including osteoblasts, chondroblasts, adipocytes and others (Pittenger et al. 1999). BMSCs are potential perfect seed cells for tissue repair, as they display unique features, such as immunoregulatory and wide source

MGF E peptide, as a special growth factor, can control cell proliferation and differentiation. Therefore, MGF E peptide may also play an important role in the regulation of biological activity of the stem cells. Collins et al. (2010) showed that treatment with MGF E peptide increased migration ability of human BMSCs. However, no further study of the role of MGF E peptide in BMSCs has been undertaken and the mechanism of migration is unclear. For this reason, we studied the effects of MGF E peptide on the proliferation, differentiation and migration of rat BMSCs (rBMSCs) and clarified the regulation mechanism. Our results showed that MGF E peptide could provide a significantly increased migration ability of rBMSCs, inhibit osteoblast differentiation and promote adipose differentiation. Receptor and signal pathway analysis indicated that MGF E peptide exhibits its functions through the Erk1/2 pathway and depends on IGF1 receptor. This investigation is helpful for understanding the function of MGF E peptide in bone homeostasis in vivo and promoting the clinical application of BMSCs.

Materials and methods

Cell culture

Fresh bone marrow was obtained from the femurs of SD rat. rBMSCs were isolated from these marrow aspirates. Briefly, bone marrow was diluted with 5 ml of DMEM/F12 medium (Gibco), centrifuged at 800 g and the supernatant was removed. The cell pellets were then resuspended in a growth medium that consisted of DMEM/F12 medium, 10% foetal bovine serum (FBS, Gibco), 1% penicillin–streptomycin (P/S), 2 mM l-glutamine (Beyotime, Jiangsu, China) and 2 ng/ml bFGF (PeproTech, Rocky Hill, NJ, USA). When rBMSCs were 90% confluent, the cells were dissociated with 0.25% trypsin containing 1 mM EDTA (Hyclone, Shanghai, China) for 1 min at 37 °C. Cells were then replated for continued passage. rBMSCs (P3) were characterised by differentiation ability assay and flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA) analysis of CD cell surface proteins: CD29 (BioLegend, San Diego, CA, USA), CD54 (BioLegend), CD11b (BioLegend) and CD45 (BioLegend). For in vitro osteoblasts differentiation, rBMSCs were seeded in a growth medium without bFGF at a concentration of 3 × 10^3 cells/cm^2. The following day (day 0), the cells were grown in osteogenic medium which consisted DMEM medium (Gibco), 10% FBS, 1% P/S, 2 mM l-glutamine, 10 nM dexamethasone (Sigma–Aldrich), 10 mM β-glycerophosphate (Sigma–Aldrich) and 0.05 mM ascorbic acid (Sigma–Aldrich). The osteogenic medium was replaced every 3 days. For in vitro adipose differentiation, rBMSCs were seeded in a growth medium without bFGF at a concentration of 1 × 10^4 cells/cm^2. When rBMSCs were 100% confluent, they were grown in adipogenic medium which consisted DMEM medium, 10% FBS, 1% P/S, 2 mM l-glutamine, 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine (Sigma–Aldrich), 10 μg/ml insulin (Sigma–Aldrich) and 200 μM indomethacin (Sigma–Aldrich). The adipogenic medium was replaced every 3 days.

For the analysis of the effect of MGF E peptide on differentiation, 50 ng/ml of MGF E peptide (Phoenix Pharmaceuticals, Catalog 033-42; Burlingame, CA, USA) was added onto rBMSCs undergoing differentiation. Due to low stability, MGF E peptide was added every 2 days. For the analysis of the effect of MGF E peptide on signal pathway, rBMSCs were subjected to serum deprivation for 12 h and then treated with 0, 30 and 50 ng/ml of MGF E peptide for 30 min. After that, rBMSCs were collected for further study.

Western blotting

rBMSCs were collected on ice, centrifuged and treated with cell-lysis buffer for extracting protein. The protein
volume was quantified using Bicinchoninic Acid Assay Kit (BCA, Beyotime). Equal volumes of protein (40–50 mg) were separated by 17% SDS–polyacrylamide gel, transferred onto PVDF membrane and processed for protein expression using specific primary antibodies at the indicated dilutions: 1:5000 phospho-Erk1/2 (Cell Signaling Technology, Beverley, MA, USA); 1:10 000 total-Erk (Cell Signaling); Col1 (Proteintech, Wu Han, China). The appropriate secondary antibody (Beyotime) was used at 1:5000 dilution. Immunoreactive signal was visualised using ECL (Millipore, Billerica, MA, USA) detection kit, according to the manufacturer’s instructions.

Proliferation assay

rBMSCs were seeded into a 96-well plate at a density of $2 \times 10^4$ cells/well. The following day (day 1), 5, 10, 30 and 50 ng/ml of MGF E peptide and IGF1 were added into different wells respectively. Cell viability was determined using Cell Counting Kit-8 (Beyotime) after 24 and 48 h following the manufacturer’s protocol. The absorbance of each well was measured with a microplate reader set at 450 nm.

Wound-healing assay

rBMSCs were seeded into a 24-well plate at a concentration of $1 \times 10^4$ cells/cm$^2$. When cells were 90% confluent, 200 µl pipette tips were used to make scratch straight wounds: 30 and 50 ng/ml of MGF E peptide were added into different wells respectively. 0 h measurements were acquired to calculate the width of the wound. After 5 and 10 h, each plate was examined by microscopy for the amount of wound closure, which was measured by the physical separation remaining between the original wound widths.

Transwell assay

We used a 10-µm-thick polycarbonate porous membrane insert with 8 µm pores (Coring Costar, Shanghai, China) to check the migration of rBMSCs. When cells were 90% confluent, they were subjected to serum deprivation for 12 h. Then the cells were detached and added to the top of the transwell insert at a concentration of $4 \times 10^5$ cells/ml. For studying the inhibitor effect of IGF1 receptor and Erk1/2, the cells were treated with PQ401 (10 µg/ml), PD98059 (50 µM) and U0126 (10 µM) for 30 min respectively before adding into the upper chamber of the transwell insert. Each top insertion contained 200 µl of cell suspension and each bottom well contained growth medium without bFGF. The concentrations of MGF E peptide in bottom well were 0, 30 and 50 ng/ml respectively. The concentration of IGF1 in bottom was 50 ng/ml. The cells migrated for 5 h and then the top inserts were removed. The cells were removed from the upper side of the membrane using a cotton bud to clear non-migratory cells. The top inserts were washed with PBS and stained with 0.1% crystal violet for 30 min. Pictures of the cell layers were obtained using a light microscope and the cell numbers using Image J software.

Alizarin red assay

For assessing the mineralised matrix, rBMSCs treated in the osteogenic medium for 14 days were stained. The cell layers were rinsed with PBS three times and fixed in 4% paraformaldehyde for 20 min at room temperature (RT). Then, the cell layers were washed with deionised water three times. The fixed cells were stained with 2% Alizarin red (Sigma) for 30 min. After washing with deionised water three times, pictures of the cell layers were obtained using light microscopy. For quantitative analysis, cells were treated with 10% (v/v) warm (50 °C) acetic acid for 30 min with shaking. Then absorbance was measured with a microplate reader set at 490 nm.

ALP activity assay

For assessing the ALP activity, rBMSCs treated in osteogenic medium for 7 days were collected and lysed by lysis buffer. Then the protein was quantified using BCA Assay Kit. ALP activity was assessed by AKP Assay Kit (Beyotime) following the manufacturer’s protocol.

Oil Red O assay

For assessing the lipid drops in adipocytes, rBMSCs treated in osteogenic medium for 14 days were stained with Oil Red O (sigma). The cell layers were rinsed with PBS three times and fixed in 4% paraformaldehyde for 20 min at RT. Then, the cell layers were washed with deionised water three times. The fixed cells were stained with Oil Red O for 10 min. To quantify staining, Oil Red O was extracted from cells with isopropanol containing 4% Nonidet P-40, and the optical density (OD) was then measured at a wavelength of 490 nm.
Adhesion assay

For cell adhesion assays, 96-well plates were coated with 10 μg/ml fibronectin (FN) overnight. The plates were blocked with 100 μl 1% BSA (Sigma) for 1 h and washed with PBS before the assay. Then 200 μl of MSC suspension (5×10^3 cells/ml) in a medium without serum were added to each well for 1 h, washed with PBS and the adherent cells were analysed by CCK-8.

Quantitative reverse transcriptase PCR

Total RNA from cells was extracted using RNApure kit (Biotek, Beijing, China) following the manufacturer’s protocol. DNase-treated RNA was reverse transcribed to generate cDNA using PrimeScript RT-PCR Kit (Takara, Dalian, China). All real-time quantitative PCRs were carried out in 25 μl reaction mixtures containing 2 μl of the cDNA preparation that are amplified with specific primers and a master mix (SYBR Green Supermix, Takara) in a CFX96TM Real-Time System (C1000 Thermal Cycler; Bio-Rad, Hercules, CA, USA). The sequences of primer are presented in Table 1.

Statistical analysis

Each experiment was carried out at least three times. Mean values and s.d. were calculated for each group, and groups were compared using t-test. Value of P<0.05 denotes a statistically significant difference.

Results

Characterisation of rBMSCs

We obtained primary BMSCs from SD rat femurs and detected differentiation capacity and phenotypic characterisation for further analysis. rBMSCs were cultured in osteogenic medium and adipogenic medium for 14 days respectively. Then osteoblast and adipose differentiation were detected by ALP, alizarin red and Oil Red O staining to find ALP activity, mineralised deposits and oil droplets. Supplementary Figure S1A (see section on supplementary data given at the end of this article) showed the ALP staining, mineralised deposits and oil droplets formation, indicating that rBMSCs had multiple differentiation potentials. Phenotypic characterisations were analysed by flow cytometry. Over 96% of cells expressed CD29, CD54 and did not express the haematopoietic marker CD45 and CD11b (Supplementary Figure S1B).

Effects of MGF E peptide on proliferation and migration of rBMSCs

To investigate the effect of MGF E peptide on cell proliferation of rBMSCs, we added different concentrations of MGF E peptide and analysed cell proliferation using CCK-8 after 24 and 48 h. As shown in Fig. 1A, 1, 5, 10, 30 and 50 ng/ml of MGF E peptide did not show obvious cell proliferation at 24 and 48 h.

Next, to explore the role of MGF E peptide in rBMSCs migration, wound healing assay, which is generally considered as a simple and reliable method to quantitatively evaluate cell motility (Liang et al. 2007), was performed. A scratch was made on a confluent cell monolayer. The migration distance represented the migration ability of rBMSCs. Figure 1C and D shows the effect of MGF E peptide on migration ability of cultured rBMSCs. After 5 h, the migratory distance of rBMSCs in MGF E peptide of 30 ng/ml increased significantly (146.0±33.4 compared with 75.2±26.6 in control cells, P=0.007). And when the concentration of MGF E peptide reached 50 ng/ml, rBMSCs also achieved a higher migration distance (187.5±30.3) than the control (P=0.009). The migration ability of rBMSCs was also increased after 10 h treatment with MGF E peptide of 30 ng/ml (P=0.002) and 50 ng/ml (P=0.038) compared with the control. The difference in cell number between 30 and 50 ng/ml was not significant (P=0.263).

To confirm wound healing migratory results, transwell assay (another method to evaluate cell migration ability) was performed to analyse the migratory function of MGF E peptide. As shown in Fig. 1E, both 30 ng/ml (P=0.002) and 50 ng/ml (P=0.003) of MGF E peptide increased the number of cells in the lower surface of the membrane (30 ng/ml=178.2±30.8, 50 ng/ml=184.2±19.4 and control=100.0±19.3). The difference in cell numbers between 30 and 50 ng/ml of MGF E peptide was also not significant (P=0.396). Therefore, a conclusion was drawn from the results of wound-healing and transwell assays, that MGF E peptide could promote rBMSCs migration significantly but in a dose-independent manner.
To analyse the difference between MGF E peptide and IGF1, we carried out proliferation and migration assays using IGF1. Unlike MGF E peptide, IGF1 with moderate concentration (10, 30 and 50 ng/ml) contributed to rBMSCs proliferation significantly in 48 h (Fig. 1B). However, the result of migration indicated that both IGF1 and MGF E peptide promoted rBMSCs migration (Fig. 1F). In addition, the migration effect of MGF E peptide was higher than the effect of IGF1 ($P < 0.032$). In conclusion, our data indicated that the effect of MGF E peptide was specific to IGF1 on BMSC. However, it is unclear whether MGF E peptide is an independent factor or an assisting factor to IGF1 action.

Effect of MGF E peptide on osteoblast and adipocyte differentiation

To investigate the function of MGF E peptide in the differentiation of rBMSCs, we analysed the expression levels of osteoblast differentiation-related genes and proteins. The expressions of three important osteoblast differentiation genes, Runx2, Alp and Col1 (Tg(Coll1a1-cre) Ikr) were measured using RT-PCR. As is shown in Fig. 2A, the expressions of Runx2, Alp and Col1 decreases in rBMSCs treated with MGF E peptide (50 ng/ml) on day 7. MGF E peptide resulted in a 20% decrease in the expression of Runx2 ($P = 0.04$), a 30% decrease in the expression of Alp ($P = 0.02$) and a 35% decrease in the expression of Col1 ($P = 0.02$). Protein analysis of ALP activity and COL1 secretion were suppressed by MGF E peptide in differentiating rBMSCs on day 7 (Fig. 2B and C). However, ALP activity with MGF E peptide did not decrease significantly on day 3 ($P = 0.056$, Fig. 2B). Moreover, the mineralisation of rBMSCs was not influenced by MGF E peptide (Supplementary Figure S2A and B, see section on supplementary data given at the end of the experiment).

Figure 1
Proliferation and migration of rBMSCs treated with MGF E peptide. (A) rBMSCs were exposed to different concentration (1, 5, 10, 30 and 50 ng/ml) of MGF E peptide for 24 and 48 h. Cell viability was measured by CCK-8. (B) rBMSCs were exposed to different concentrations (1, 5, 10, 30 and 50 ng/ml) of IGF1 for 24 and 48 h. Cell viability was measured by CCK-8. (C) Scratch wound-healing assay of MGF E peptide (0, 30 and 50 ng/ml) on rBMSCs. The cells were subjected to in vitro scratch assay with images captured at 0, 5 and 10 h using a microscope. The migration ability was measured by quantifying the difference in distance between the two edges of the scratch at different time points. (D) Representative images of wound healing of rBMSCs. (E) Transwell assay of MGF E peptide (0, 30 and 50 ng/ml) on rBMSCs. After 5 h migration, the number of cells on the upper side of the membrane represented the number of migration cells. (F) Transwell assay of MGF E peptide (50 ng/ml) and IGF1 (50 ng/ml) on rBMSCs. Scale bars: 100 μm. $n \geq 3$. *$P < 0.05$; **$P < 0.01$. 

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These data suggested that MGF E peptide can significantly suppress osteoblast differentiation and reduce extracellular matrix protein secretion by reducing osteogenic genes expression.

The osteogenesis and adipogenesis in rBMSCs rely on the dynamic balance. The osteoblast differentiation process always inhibits adipose differentiation. Hence, we assessed the effect of MGF E peptide on rBMSCs adipogenic lineage commitment and analysed the expression levels of two key adipogenesis-related genes, Pparγ and Fabp4. When rBMSCs were treated with adipogenic medium and 50 ng/ml of MGF E peptide on day 7, the expression of both PPARγ \((P=0.016)\) and FABP4 \((P=0.004)\) was increased by 144 and 139%, respectively, compared with the control (Fig. 3A). Supporting the mRNA expression result, Oil Red O staining and quantification results showed that MGF E peptide could promote lipid deposition accumulation during MSCs adipose differentiation (Fig. 3B and C). Together, the results indicated that MGF E peptide played a crucial role in the balance between osteogenesis and adipogenesis of rBMSCs.

Mechanism of MGF E peptide function on the migration of rBMSCs

There is a close relation between adhesion and migration. The migration of adherent cells is regulated by changes...
in the affinity of cells to extracellular matrix. In this study, we wonder whether MGF E peptide regulates rBMSCs adhesion, thereby increasing migration ability. Figure 4A shows that 30 ng/ml \((P=0.235)\) and 50 ng/ml \((P=0.306)\) MGF E peptide did not cause a significant difference in the number of adhering cells, comparison with the control. Then, we analysed IGF1/Erk1/2, which is involved in cell migration in many cell types. We checked whether MGF E peptide would increase phosphorylation of ERK1/2 in rBMSCs. The cells were treated under different concentrations (30 and 50 ng/ml) of MGF E peptide for 30 min and then the levels of phosphorylation-Erk1/2 were analysed. Immunoblotting results showed that 30 ng/ml \((P=0.006)\) and 50 ng/ml \((P=0.013)\) MGF E peptide could activate phosphorylation-Erk (Fig. 4B and C); 30 and 50 ng/ml MGF E peptide exhibited an equal activating effect \((P=0.150)\). However, when cells were treated with IGF1 receptor inhibitor (PQ401), the level of phosphorylation-Erk1/2 was suppressed and downregulated to the normal level \((P=0.104)\). These results indicate that the MGF E peptide activated phosphorylation-Erk1/2 is dependant on IGF1 receptor. On the basis of the result, further studies were focused on whether MGF E peptide activates the Erk1/2 pathway to promote rBMSCs migration. So we used inhibitors of Erk1/2 (PD98059 and U0126) and IGF1 receptor (PQ401).
to analyse the mechanism of the MGF E peptide. As shown in Fig. 4D, when cells were pretreated with PD98059 ($P=0.079$) or U0126 ($P=0.106$), MGF E peptide treatment did not significantly increase rBMSCs migration ability. IGF1 receptor inhibitor ($P=0.083$) also suppressed the migration effect of MGF E peptide completely. From these results, we know that the MGF E peptide promote migration dependent on the IGF1 receptor via the Erk1/2 signal pathway.

Discussion

In previous studies, MGF E peptide has been reported to promote cell proliferation in C2C12 (Yang & Goldspink 2002). However, our results showed that it had no significant proliferation effect on rBMSCs, which was similar to Collins’ findings (Collins et al. 2010).

Both wound-healing and transwell assays showed that MGF E peptide provided a significant increase in migration ability. There is a close relationship between cell adhesion and migration in many types of cells (Imhof & Aurrand-Lions 2004, Vicente-Manzanares et al. 2009). For example, the adherence of endothelial cells to the extracellular matrix is crucial for proliferation and migration (Vicente-Manzanares et al. 2009). Our results from the adhesion assay indicated that the migration effect of MGF E peptide did not result from altering cell adhesion. Then we focused on the Erk1/2 pathway, which is a key signal way to regulate cell proliferation and differentiation. Erk1/2 has been shown to be involved in the migration of numerous cell types in response to growth factors (Pintucci et al. 2002, Huang et al. 2004, Motobayashi et al. 2009, Kashyap et al. 2011).

In our study, MGF E peptide activated phosphorylation-Erk1/2 via IGF1 receptor. Further research of Erk1/2 inhibitors confirmed that the Erk1/2 signal pathway is involved in the migration effect of the MGF E peptide. When the Erk1/2 signal pathway was blocked by PD58059 or U0126, MGF E peptide treatment could not increase migration ability significantly, suggesting that MGF E peptide mediates migration via the Erk1/2 signal pathway. In previous study, it remained unclear whether MGF E peptide performed its function through IGF1 receptor. Different reports suggest contradictory conclusions (Mills et al. 2007a,b, Brisson & Barton 2012). Our result for IGF1 receptor inhibitor indicates that MGF E peptide depends on IGF1 receptor to cause the migration effect. Conclusively, MGF E peptide activates Erk1/2 signal pathway relying on IGF1 receptor, thereby promoting cell migration. A similar signal mechanism has been previously described in C2C12 treated with MGF E peptide (Brisson & Barton 2012). The migration effect of MGF E peptide on BMSCs is important for BMSCs homing to specific tissues. For example, tissue damage in the heart is associated with a local increase in MGF, which may recruit MSCs to participate in myocardial repair (Stavropoulou et al. 2009).

MGF E peptide has been reported to delay myotubule fusion in C2C12 (Dluzniewska et al. 2005) and suppress osteoblast differentiation and mineralisation in the MC3T3 cell line (Xin et al. 2012). In our experiments, three important osteoblast differentiation genes ($\text{Alp}$, $\text{Runx2}$ and $\text{Col1}$) were all downregulated in response to MGF E peptide. ALP activity and COL1 secretion were also suppressed by MGF E peptide. Runx2 is essential for osteoblast differentiation and the maintenance of bone (Ducy et al. 1997). COL1, containing Runx2-binding sites in promoter regions, is an important osteoblast extracellular matrix protein. High ALP activity is crucial for bone mineral formation (Matsubara et al. 2008). There is a balance between adipogenesis and osteogenesis and inhibition of osteogenesis may promote adipogenic differentiation in BMSCs (Ye et al. 2012). In our results, besides inhibiting osteogenic differentiation, MGF E peptide also enhanced adipogenesis by upregulating $\text{Ppary}$ and $\text{Fabp4}$. However, the inhibiting effect of MGF E peptide on osteogenesis is opposite to the function of IGF1Ea (Xian et al. 2012). Previous studies have shown that IGF1Ea is able to activate the MAPK/Erk1/2 and PI3k/AKT/mTOR signal pathway through IGF1 receptor, and it increased osteoblast differentiation and maintenance of bone mass through activating the PI3k/AKT/mTOR pathway. Some reports suggested that MGF E peptide could only cause MAPK/Erk1/2 and PI3k/AKT activation but not PI3k/AKT (Mills et al. 2007a,b, Philippou et al. 2009, Armakolas et al. 2010, Brisson & Barton 2012). This difference may lead to an opposite osteogenic differentiation effect between IGF1Ea and MGF E peptide. It is reported that MGF E peptide caused IGF1 receptor internalisation, which is responsible for activating p-Erk1/2 (Chow et al. 1998, Pfeffer et al. 2009). MGF E peptide may activate the MAPK/Erk1/2 signal pathway through this way. Due to lack of understanding, further studies are needed to verify the role of IGF1 receptor in response to MGF E peptide.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/JME-13-0157.
Declaration of interest
The material is original research, has not been previously published and has not been submitted for publication elsewhere while under consideration. The authors of this article declare they have no conflicts of interest.

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References

Brisson RK & Barton ER 2012 Insulin-like growth factor-I E-peptide activity is dependent on the IGF-I receptor. PLoS ONE 7 e45588. (doi:10.1371/ journal.pone.0045588)


Collins JM, Goldspink PH & Russell B 2010 Migration and proliferation of human mesenchymal stem cells is stimulated by different regions of the mecano-growth factor prohormone. Journal of Molecular and Cellular Cardiology 49 1042–1045. (doi:10.1016/j.ymcel.2010.09.017)

Dai Z, Wu F, Yeung EW & Li Y 2007 Insulin-like growth factor 1 enhances the migratory capacity of mesenchymal stem cells. Biochemical and Biophysical Research Communications 356 780–784. (doi:10.1016/j.bbrc.2007.03.049)


Liu J, Baker J, Perkins A, Robertson EJ & Efstratiadis A 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (lgf-1) and type 1 IGF receptor (lgf1r). Cell 75 59–72.


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