Metformin promotes the proliferation and differentiation of murine preosteoblast by regulating the expression of sirt6 and oct4

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Osteopenia, osteoporosis and bone salt metabolism disorder are common diseases in the aged and diabetics. From case reports of patients with T2DM, we have observed that metformin can decrease risk of bone fracture and promote bone formation. However, the underlying mechanism of metformin’s effect on bone metabolism remains unknown. In our research, we show that metformin can promote proliferation of murine preosteoblast by regulating AMPK-mTORC2 and AKT-mTORC1 signaling axis. Furthermore, we have observed that metformin can promote SIRT6 expression before and during differentiation of murine preosteoblast. The interaction between SIRT6 and NF-κB is highly important in osteoblast differentiation just as the relationship between OPG and RANKL in the process of bone formation. During differentiation, we show that SIRT6 inhibits phosphorylation of NF-κB and that OPG increases while RANKL decreases in HG groups. In addition, ablation of sirt6 in mice causes phosphorylation of NF-κB at high-levels and RANKL increases slightly in femur bone cells. However, other bone formation marker proteins such as RUNX2, OSTERIX and OPG appear at low-levels in sirt6 KO mice. It has been confirmed that downregulation of OCT4 is critical incident in the differentiation of embryonic stem cells. Fortunately, we observe that SIRT6 can suppress OCT4 expression in murine preosteoblast and the expression of OCT4 is at high-level in sirt6 KO mice. Taken together, this study’s results illuminate metformin’s effect on bone metabolism under HG condition and help to elucidate why metformin can promote bone fracture healing of patients with T2DM.

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1. Introduction

Metformin is a first-line anti-diabetic drug for treating patients with T2DM [1]. It was reported that metformin improves glucose metabolism in hyperglycemia via stimulating AMPK complex [2,3]. It has also been confirmed that AMPK complex has three different subunits that are specifically expressed in certain tissues or organs.

For example, the AMPKα subunit is highly expressed in bone tissue, primary osteoblasts, and osteoclasts as well as in a number of bone cell lines [4–7]. Thus, activation of AMPKα by metformin influences bone metabolism either directly or indirectly [8,9].

Previous studies showed that AKT played an important role in regulating glucose uptake into muscle and fat cells through stimulating the translocation of GLUT4 to the plasma membrane [10–12]. Use of alanine mutants clearly show that AKT-Thr308 and Ser473 can be phosphorylated independently of each other [13]. The mechanism of AKT in osteocyte is still unknown. There are notably few reports describing the relationship between metformin and AKT in bone metabolism.

SIRT6, which is a member of Sir2unis family and mainly expressed in nucleus, is indispensable in keeping genome stabilization, DNA repair and gene expression programs [14]. On the grounds of previous investigations, we have known that SIRT6 interacts with NF-κB [15] in bone metabolism and that the expression of NF-κB is essential in normal skeletal remodeling and bone homeostasis by controlling the differentiation of osteoprogeni-
Fig. 1. Metformin promotes proliferation and stimulates insulin-independent glucose uptake in murine preosteoblasts. (A, B) Extracellular glucose concentration changes of MC3T3 E1 under NG and HG conditions measured after culturing for 3 days. (C) RT-qPCR analysis of glut1–4 mRNA relative expression on MC3T3 E1 after metformin treatment for 48 h. (D) GLUT1 expression detected under NG conditions after metformin treatment for 48 h and analyzed by Image J software. All error bars represent SEM. Statistical analysis measured by paired t-test (one-tailed) between groups without metformin under NG and HG condition. Statistical analysis measured by one-way ANOVA among groups with different metformin concentration under NG and HG condition. *P < 0.05; #P < 0.01; ***P < 0.005; ##P < 0.001.

The expression of GLUT1 in cells treated with metformin was analyzed by RT-qPCR and western blotting. The results showed that metformin increased the expression of GLUT1. The effect of metformin on GLUT1 expression was dose-dependent, with higher concentrations of metformin leading to higher expression levels.

Furthermore, Etchebaray, J. P et al. reported that SIRT6 was a vital regulator in the differentiation of embryonic stem cells mediated by suppressing expression of oct4, sox2 and nanog [18]. However, whether metformin action on SIRT6 precedes inhibition of OCT4 expression is still unclear. Therefore, research on the relationship between metformin and SIRT6 may help to explain the metformin's effect on bone fracture of patients with T2DM and to elucidate molecular action mechanism of metformin.

2. Materials and methods

2.1. Chemicals and regents

D-glucose (dextrose) anhydrous and 4% paraformaldehyde purchased from Amersco Life Science Company. (Amersco, China). β-glycerophosphate disodium and vitamin C purchased from Biodee