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High glucose downregulates the effects of autophagy on osteoclastogenesis via the AMPK/mTOR/ULK1 pathway

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ABSTRACT

Diabetes is a chronic disease that disrupts the balance between bone formation and bone desorption, which can lead to osteoporosis, increasing the risk of fracture. However, compared with osteoblasts, the biological effects of hyperglycemia on osteoclastogenesis remain to be elucidated. Therefore, we investigated the impact of glucose at different concentrations (5.5, 10.5, 15.5, 20.5, 25.5, and 30.5 mM) on osteoclastogenesis using RAW264.7 cells. Cell proliferation was measured with the cell counting kit-8 assay, and osteoclastogenesis was detected with tartrate-resistant acid phosphatase staining and bone resorption assays, as well as protein cathepsin K expression. Compound C, the AMP-activated protein kinase (AMPK) pathway inhibitor, was used to examine the relationship between the AMPK/mTOR/ULK1 signaling pathway and autophagy in osteoclasts. Autophagy was evaluated with transmission electron microscopy and immunofluorescence microscopy and associated proteins were detected with western blotting. The pharmacological autophagic reagents bafilomycin A1, 3-methyladenine, and rapamycin were used to determine the effect of autophagy on osteoclastogenesis. Our results showed that glucose negatively affected osteoclast formation and function but did not affect the proliferation of RAW264.7 cells. Suppression of the AMPK/mTOR/ULK1 signaling axis decreased autophagy in glucose-mediated osteoclast. Furthermore, High levels of glucose decreased autophagy level in osteoclasts. Additionally, interfering with autophagy affected osteoclast formation and function. These findings clarify the mechanisms underlying the effects of glucose-mediated osteoclastogenesis and will help identify novel therapeutic strategies for the protection of skeletal health in diabetic osteoporosis.

1. Introduction

The prevalence of diabetes, one of the largest global issues of the 21st century, has increased to up to 8.8% of adults worldwide. Without appropriate disease management, diabetes can lead to complications such as cardiovascular disease, kidney failure, and nerve disease [1]. It is also being increasingly recognized that diabetes increases the risk of osteoporotic fracture [2]. Hyperglycemia, a common features of diabetes mellitus, breaks the dynamic balance of bone homeostasis by affecting osteoblasts and osteoclasts, resulting in osteoporosis [3].

Osteoclasts are a type of multinucleated cell which play a key role in bone resorption, therefore, increasing the number and promoting the function of osteoclasts can aggravate osteoporosis. In the previous studies of Wittrant et al. [4] and Xu et al. [5] suggested that high glucose negative regulate the differentiation and function of osteoclasts. However, the opposite results were observed in the other studies of Catalfamo et al. [6] and Larsen et al. [7], which indicated hyperglycemia *in vitro* promotes the differentiation and capacity for bone resorption of osteoclasts derived from murine models, chicken and individuals. In summary, it is controversial that high glucose inhibits or stimulates the differentiation and function of osteoclasts.

As a giant multinucleated cell, osteoclasts have high energetic requirements in the process of both differentiation from promonocytic precursors in marrow and bone resorption. It seems that glucose is the primary extracellular energy source to generate the

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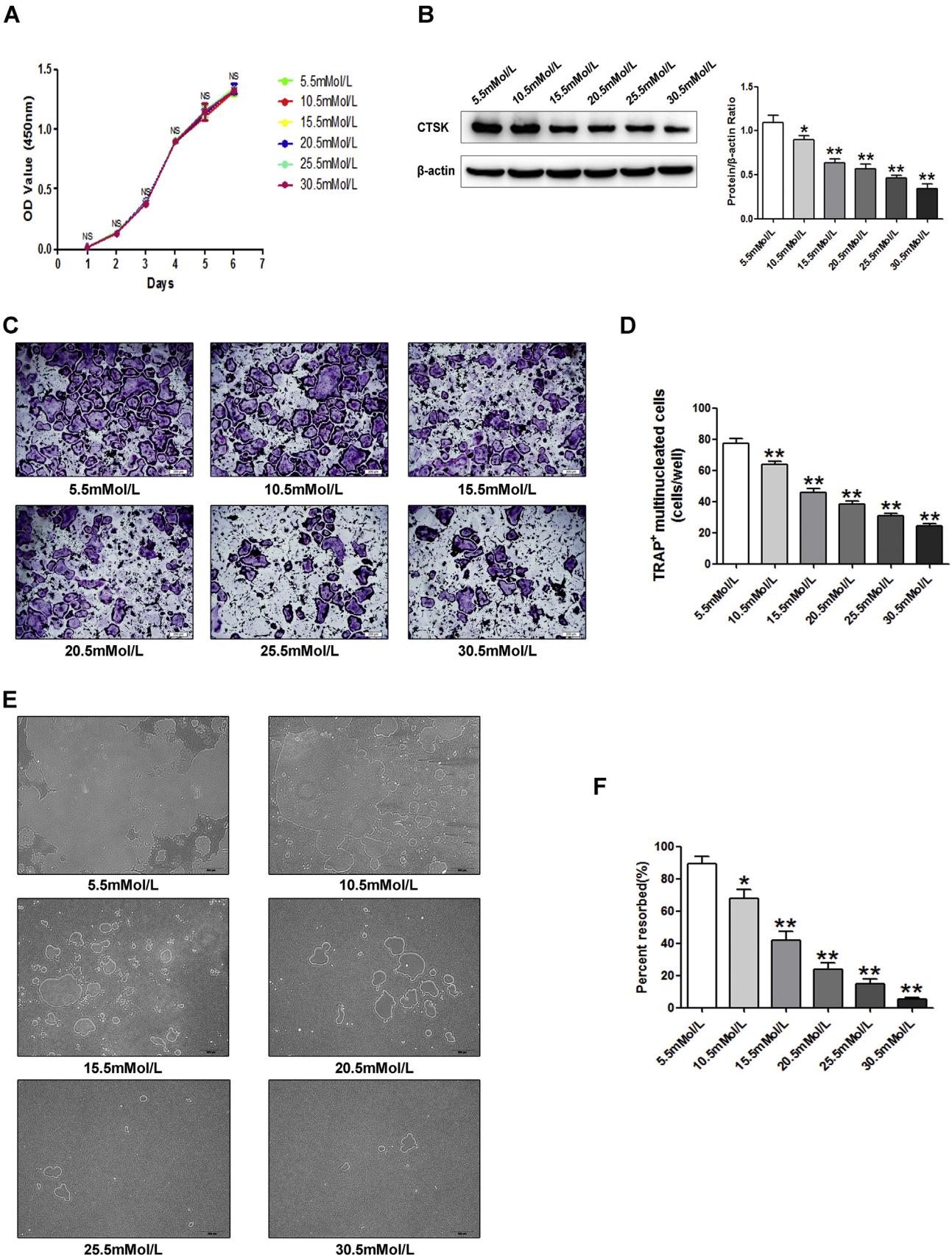

Fig. 1. Glucose had dose-related effects on the formation and function of osteoclast. RAW264.7 cells were treated with different concentrations of glucose in the absence or presence of M-CSF (30 ng/L) and RANKL (50 ng/L). The culture medium was exchanged every two days. A. RAW264.7 cells were treated with glucose at various concentrations in the absence of M-CSF and RANKL from 1 to 6 days, and cell viability was measured using CCK-8 assay at the indicated times. There was no significant difference between every two groups. Data

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