Tumor Necrosis Factor Alpha Stimulates Proliferation of Dental Pulp Stem Cells via Akt/Glycogen Synthase Kinase-3β/Cyclin D1 Signaling Pathway

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Abstract

Introduction: It has been widely accepted that dental pulp stem cells (DPSCs), which are a class of self-renewal and differentiation potential of adult stem cells, play an important role in the repair procession of pulp's inflammation. We investigated whether tumor necrosis factor alpha (TNF-α) could induce the proliferation of DPSCs and clarified the potential mechanism of this proliferation. **Methods:** Cell Counting Kit-8 assay (Dojindo Laboratories, Mashiki-machi, Kumamoto, Japan) and 5-ethynyl-2′-deoxyuridine–based proliferation assays were determined to investigate various concentrations or hours of TNF-α inducing a cell number change of DPSCs. Next, flow cytometry analysis was performed to investigate the main cell cycle phase process of DPSCs. Furthermore, the signaling pathway of TNF-α–induced proliferation of DPSCs was analyzed using Western blot analysis. Then, inhibitors were added to confirm the mechanism of this signaling pathway. **Results:** TNF-α induced the proliferation of DPSCs in a dose- and time-dependent manner. Cyclin D1, which controlled the cell cycle process from the G1 to the S phase, was up-regulated by TNF-α in a time-dependent manner, whereas its overexpression alone increased DPSC proliferation. Furthermore, TNF-α was capable of inducing Akt/GSK-3β signaling pathway activation. Blockage of phosphoinositide 3-kinase/Akt by their kinase or genetic inhibitors could significantly reduce TNF-α–induced proliferation of DPSCs. **Conclusions:** This study confirmed that TNF-α induced the proliferation of DPSCs by regulating the Akt/GSK-3β/cyclin D1 signaling pathway and then provided a suitable number for the requirements of cell differentiation. *(J Endod 2015;41:1066–1072)*

Key Words

Akt, cyclin D1, dental pulp stem cells, glycogen synthase kinase-3β, proliferation, tumor necrosis factor-alpha

Oral environments are susceptible to bacterial contamination, and these infections can easily cause periodontal disease. Periodontal disease has a high incidence and is the main reason for tooth loss (1, 2). It is characterized by the formation of periodontal pockets and absorbed alveolar bone and can cause periodontal tissue inflammation and destruction. Dental pulp stem cells (DPSCs) are a class of adult stem cells present in the pulp that have the potential of self-renewal and multilineage differentiation (3–5). As a kind of mesenchymal stem cells, DPSCs can proliferate and differentiate into odontoblastlike cells under appropriate conditions (6). These cell lines include osteo/odontogenic, adipogenic, neurogenic, chondrogenic, and myogenic (7–9). In the process of periodontal regeneration, DPSCs are in an inflammatory microenvironment in which proliferation and differentiation processes are likely to be affected by a variety of inflammatory cytokines. Among these cytokines, the most important one is tumor necrosis factor alpha (TNF-α). It can trigger a series of intracellular molecular events and multiple molecular signaling pathways as well as initiate the cell proliferation of DPSCs. Then, DPSCs differentiate into many cell types to repair dental pulp after injury as a part of the innate immune response (10–12). The ultimate goal of cell proliferation and differentiation of DPSCs is to establish periodontal tissue and restore its structure and function. Furthermore, DPSCs can be easily isolated from the freshly extracted teeth. Their simplicity and convenience of isolation, lack of ethical controversy, and low immunogenicity make them useful clinically (13, 14). There is a growing concern regarding DPSCs as seed cells for tissue engineering research (15, 16). Therefore, the application of DPSCs brings a gospel for oral problems. However, the molecular mechanisms underlying the regulation of proliferation remain unclear. Thus, further study is needed.

Cyclins are essential positive regulation components of the cell cycle machinery. Among them, cyclin D1 is a multifunctional oncoprotein that regulates the G1 to S phase transition of the cell cycle (17). The phosphoinositide 3-kinase/protein kinase B (Akt) signaling pathway plays an important role in the regulation of cell proliferation, apoptosis, differentiation, migration, and metabolism (18) and is also known to play a major role in cell cycle progression during the G1/S transition. Several Akt's substrates including glycogen synthase kinase (GSK)-3β and forkhead transcription factors are involved in the cell cycle regulation. GSK-3β is considered to regulate cell cycle progression by phosphorylation of cyclin D1 C-terminal residues Thr-286 (19–21).

Based on these backgrounds, analyzing the mechanism of proliferation was inevitable in understanding the features of DPSCs. In this study, we attempted to investigate

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whether TNF-α could induce the proliferation of DPSCs and their major signaling pathway.

Materials and Methods

Cell Culture and Treatment

Normal human impacted third molars were collected from patients 13–23 years of age (n = 9) after giving informed consent; this study was approved by the Ethics Committee of People's Hospital of Zoucheng. All subjects were free of carious lesions and oral infection. We isolated DPSCs by cleaning the tooth surface, cutting around the cementoenamel junction using sterilized dental fissure burs, and then opening to reveal the pulp chamber. The pulp was digested in a solution of 3 mg/mL collagenase type I for 1 hour at 37°C. Single-cell suspensions were obtained by passing the digested tissues through a 70-μm cell strainer (BD Falcon, Shanghai, China). Cell suspensions of dental pulp were seeded into 25-cm^2 culture dishes and cultured in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (Sigma-Aldrich, St. Louis, MO) at 37°C under 5% CO_2 condition. The medium was changed every 3 days. Cells at passages 3 or 4 were used for further experiments.

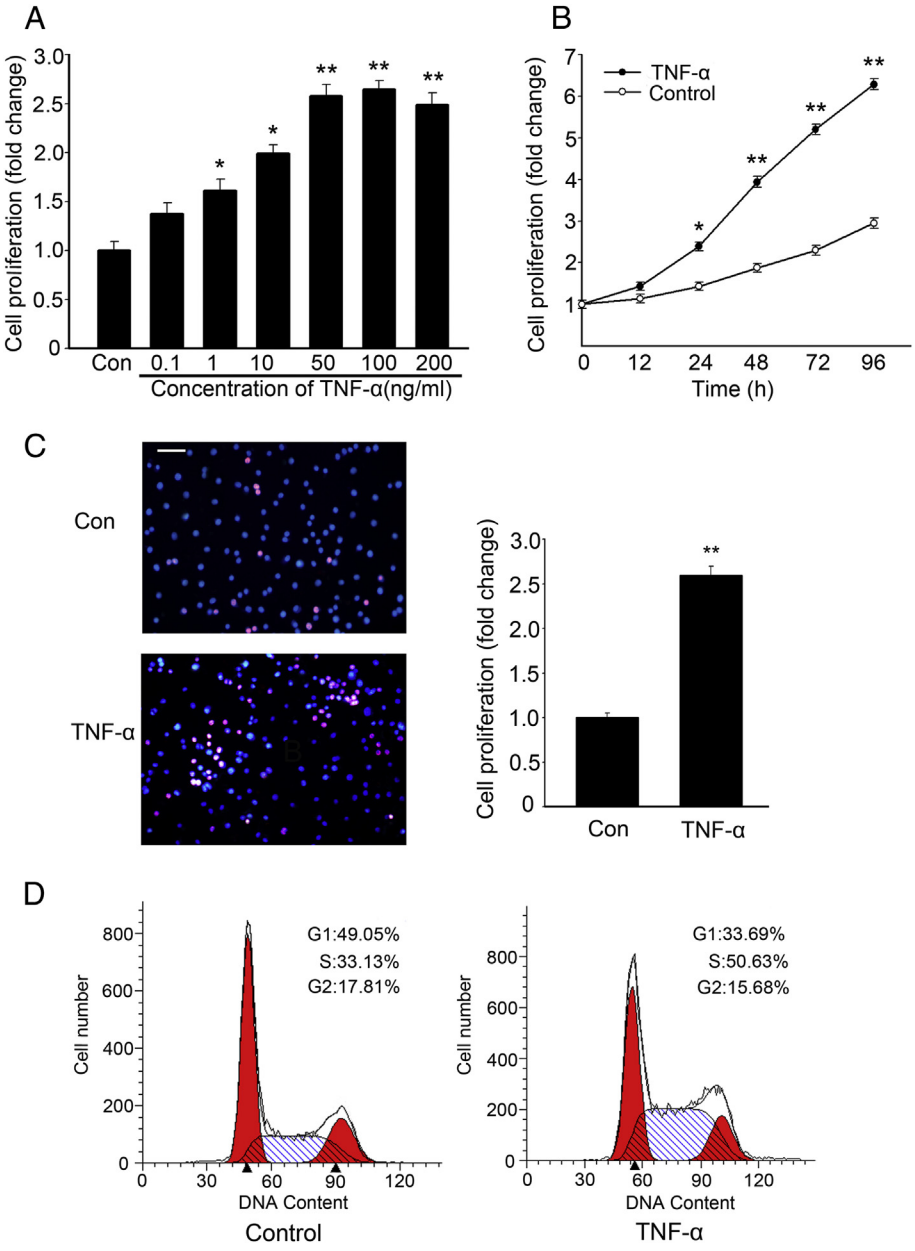

Figure 1. TNF-α stimulated the proliferation of DPSCs. (*A*) DPSCs were treated with various concentrations (0, 0.1, 10, 50, 100, and 200 ng/mL) of TNF-α for 48 hours. The cell number of DPSCs was detected by the CCK-8 assay. (*B*) DPSCs were treated with 50 ng/mL TNF-α or the same volume of DMEM (as controls) for various hours (0, 12, 24, 48, 72, and 96 hours). The cell number of DPSCs was detected by the CCK-8 assay. (*C*) After treated with DMEM (control) or 50 ng/mL TNF-α for 48 hours, cells were assessed by the EdU-based proliferation assay. Proliferating cells were detected with EdU+ (*violet*). Nuclear was counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (*blue*). (*D*) Flow cytometry analysis showed the cell cycle phase of DPSCs after being treated with DMEM (control) or 50 ng/mL TNF-α for 48 hours. Values are means ± standard error of the mean (n = 3). *P < .05, **P < .01, significantly different from control group. Scale bars = 50 μm.