MP039

ERK1/2 SIGNALING MEDIATED NARINGIN-INDUCED OSTEOGENIC DIFFERENTIATION OF THE IMMORTALIZED HUMAN PERIODONTAL LIGAMENT STEM CELLS

Kai Wei[1,2], Yuansheng Xie[1], Tianyu Chen[3], Bo Fu[1], Shaoyuan Cui[1], Yan Wang[3], Guangyan Cai[1], Xiangmei Chen[1]
1 Department of Nephrology, Chinese PLA General Hospital, Chinese PLA Institute of Nephrology,
State Key Laboratory of Kidney Diseases, National Clinical Research Center for Kidney Diseases, Beijing 100853, China
2 Medical College, Nankai University, Tianjin 300071, China
3 Guanghua School of Stomatology, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Stomatology, Guangzhou 510080

INTRODUCTION AND AIMS

Periodontal ligament stem cells (PDLSCs) are promising tools for the investigations of cell differentiation and bone regeneration. However, the limited life span significantly restricts their usefulness. In addition, naringin had been reported to promote the osteogenesis of PDLSCs, but the underlying mechanisms hasn't been revealed yet. Herein, we aim to establish an immortalized PDLSC cell line and to investigate the potential mechanisms of the osteogenic differentiation of immortalized PDLSCs induced by naringin.

METHODS

Primary PDLSCs were extracted from healthy premolars of 10 adults for orthodontic purposes. All participants provided informed consent for the collection and use of their tissues. Bmi1 gene was introduced into PDLSCs (PDLSC-Bmi1) by lentiviral transduction. Real time PCR was used to determine the expression of Bmi1, telomerase reverse transcriptase gene (TERT) and osteogenesis related genes. Bmi1, $p16^{INK4a}$ and ERK1/2 were studied by immunoblotting. Senescence-associated β-galactosidase (SA-βGal) was assayed by SA-βGal staining. Mesenchymal stem cell (MSCs) surface markers were detected by flow cytometry. The mesenchymal and epithelial markers were performed by immunofluorescence staining. The mineralized nodules were stained by Alizarin Red S.

RESULTS

1. The PDLSC-Bmi1 acquired a slower aging rate. Compared with PDLSCs, the expression of Bmi1 and TERT increased dramatically in PDLSC-Bmi1 (Fig 1A-C). For PDLSCs at P20, the senescence related $p16^{INK4a}$ and SA-βGal accumulated significantly (Fig 1D and E). In contrast, the expression of $p16^{INK4a}$ was obviously less in the PDLSC-Bmi1 at P20 (Fig 1D), and few cells were positive for SA-βGal (Fig 1F). Furthermore, the PDLSC-Bmi1 could proliferate for more than 30 passages and the morphology maintained almost the same as the PDLSCs at early passages (Fig 1G and H).

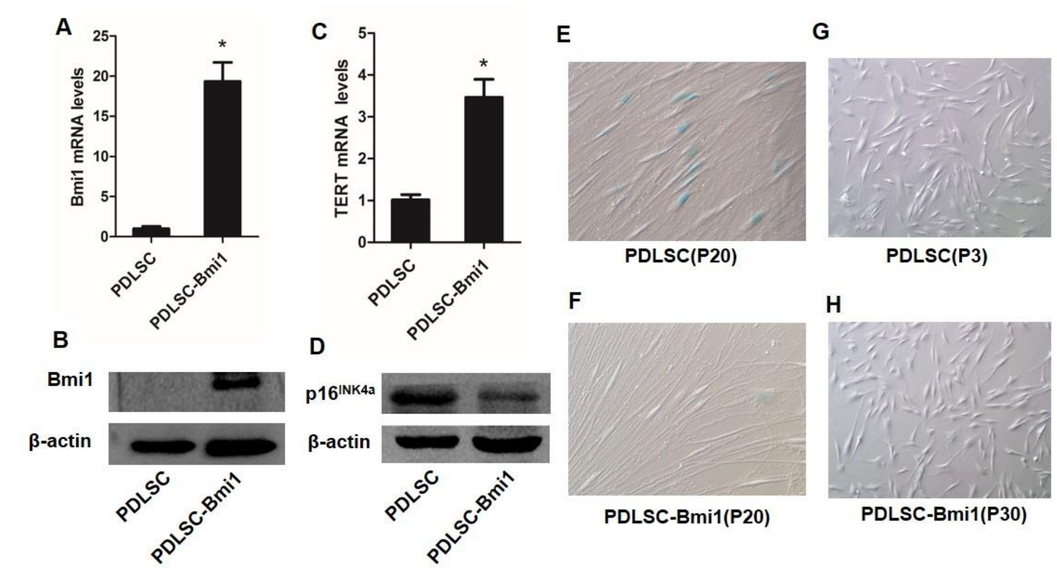

Figure 1. The senescence of PDLSC and PDLSC-Bmi1. (A-B) Expression of Bmi1in PDLSC and PDLSC-Bmi1. (C) Expression of TERT in PDLSC and PDLSC-Bmi1. (D) Expression of $p16^{ink4a}$ in PDLSC and PDLSC-Bmi1. (E-F) SA-βGal staining of PDLSC and PDLSC-Bmi1, 320x. (G-H) Morphology of PDLSC and PDLSC-Bmi1 at different passages, 200x.

2. The PDLSC-Bmi1 maintained the phenotype and functions of primary cells. More than 98% of PDLSC-Bmi1 were positive for CD44, CD73, CD90 and CD105; while less than 2% of them were positive for hematopoietic surface markers. More than 30% of the PDLSC-Bmi1 were positive for CD146, a typical marker of PDLSCs (Fig 2A). The PDLSC-Bmi1 were positive for vimentin but negative for E-cadherin (Fig 2B and C). The PDLSC-Bmi1 also had osteogenic and adipogenic potentials (Fig 2D and E).

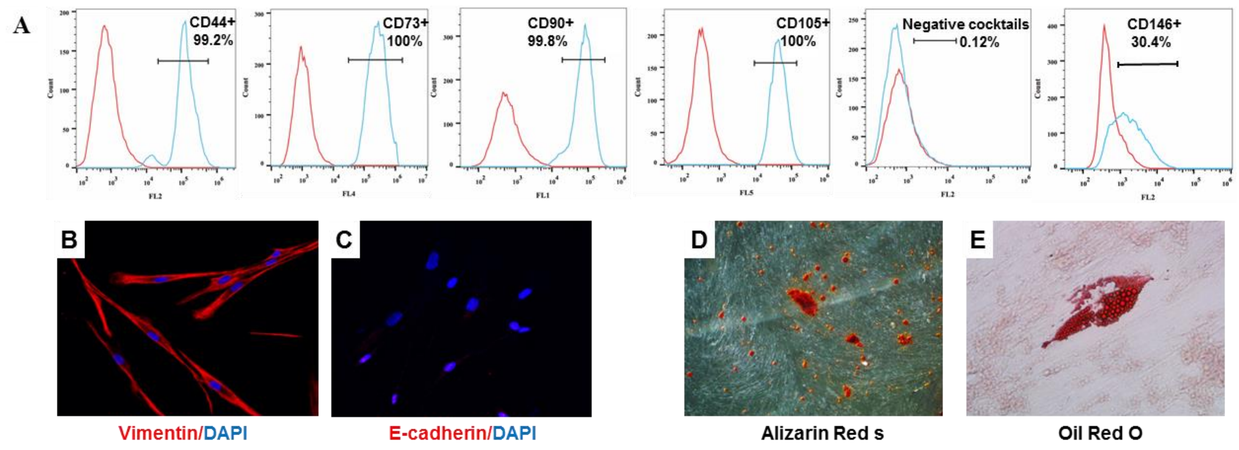

Figure 2. Phenotype and differentiation potentials of PDLSC-Bmi1. (A) Flow-cytometry for MSC surface markers and PDLSC marker. (B-C) Immunofluorescence staining for vimentin and E-cadherin, 400x. (D) Alizarin red s staining for mineralized nodules, 200x. (E) Oil red o staining for lipid drops, 400x.

3. Naringin promoted the osteogenesis of PDLSC-Bmi1 cells. The ALP activity (Fig 3A), expression of Runx2 and OCN (Fig 3B and C) and formation of mineralized nodules (Fig 3D-F) were significantly increased after the administration of naringin.

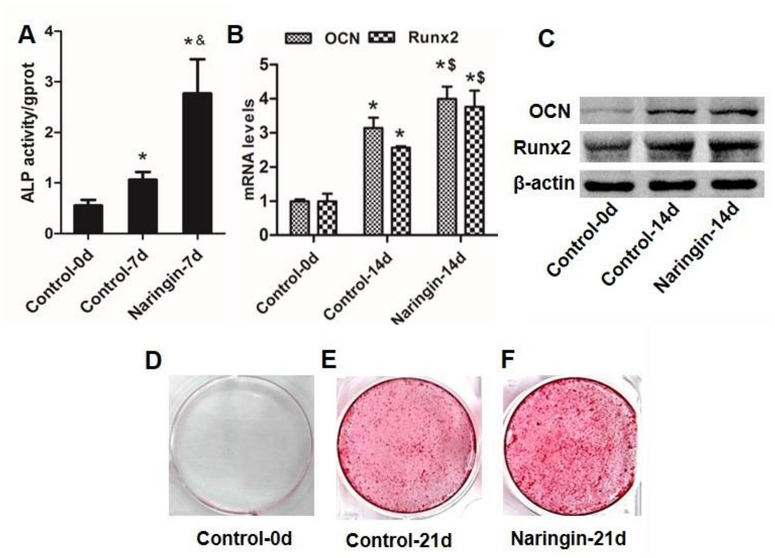

Figure 3. Naringin promoted the osteogenesis of PDLSC-Bmi1 cells. (A) Naringin increased the ALP activity.(B-C) Naringin increased the expression of Runx2 and OCN. (D-F) Naringin increased the formation of mineralized nodules.

4. ERK1/2 mediated the effects of naringin on PDLSC-Bmi1 cells. Naringin activated the phosphorylation of ERK1/2 in a time-dependent manner (Fig 4A). The ERK1/2 inhibitor, PD98059, prevented the activation of ERK1/2 (Fig 4B) and attenuated the increase of ALP activity (Fig 4C), expression of Runx2 and OCN (Fig 4D and E) and the formation of mineralized nodules induced by naringin (Fig 4F-H).

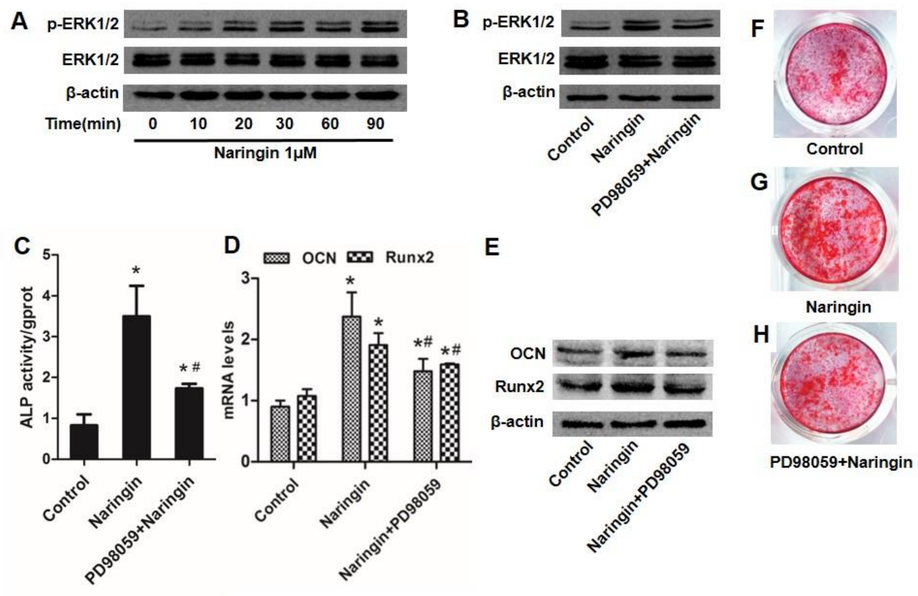

Figure 4. ERK1/2 mediated the effects of naringin on PDLSC-Bmi1 cells. (A-B) The phosphorylation of ERK1/2 under different treatments. (C) PD98059 attenuated the increase of ALP activity. (D-E)) PD98059 attenuated the increase the expression of Runx2 and OCN. (F-H)) PD98059 attenuated the increase the formation of mineralized nodules.

CONCLUSIONS

The overexpression of Bmi1 extended the life span of PDLSCs without disturbing their phenotype and biological functions, and that naringin promoted the osteogenesis of immortalized PDLSCs partially through the ERK1/2 signaling pathway.

DOI: 10.3252/pso.eu.54ERA.2017