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Role of PI3K/AKT Signaling Pathway in Proliferation, Migration and Odontogenic Differentiation of Human Dental Pulp Stem Cells

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Abstract: We aimed to study the role of the PI3K/AKT signaling pathway in the proliferation, migration and odontogenic differentiation of human dental pulp stem cells (hDPSCs). hDPSCs were cultured in vitro and treated with LY294002 (LY), an inhibitor of the PI3K pathway. Cell proliferation and migration were detected by CCK8 assay and scratch assay respectively. The levels of PI3K and AKT pathway downstream proteins AKT, phosphorylated AKT (p-AKT) as well as odontogenic differentiation marker DSPP were measured by Western blot at 6, 12, 24, 48 and 72 h. Blank control, mineralization induction, LY and mineralization induction + LY groups were set. After 14 days of mineralization induction, the formation of mineralized nodules was detected by alizarin red staining. At 24, 48 and 72 h, the optical densities of LY group were significantly lower than those of control group (P<0.05). Compared with 0 h after scratching, the cells in each group migrated at 24 h. Compared with control group, the number of migrating cells in LY group was significantly lower. After 14 days of mineralization induction, the mineralization induction group had most mineralized nodules, with the highest density. The protein levels of p-AKT and AKT remained almost unchanged at 0, 6, 12, 24, 48 and 72 h. After addition of LY294002, the protein levels of p-AKT decreased from 6 to 72 h, that of AKT did not change significantly, and that of DSPP reduced. The PI3K/AKT signaling pathway can promote the proliferation, migration and odontogenic differentiation of hDPSCs.

Key words: AKT, Dental pulp, PI3K, Signaling pathway, Stem cell

Introduction

Dental pulp stem cells (DPSCs) are adult stem cells that exist in dental pulp tissues, with multi-directional differentiation potential and self-renewal ability1. They were obtained as early as 2000 by Gronthos et al. through in vitro culture of adult third molar pulp cells2. DPSCs can differentiate into osteoblasts, chondroblasts, adipocytes and neuroblasts in various induction microenvironments3. They have been widely used in the regeneration of oral and maxillofacial tissues, dental pulp tissues, teeth, nerves and salivary gland tissues4. DPSCs have become an important source of cells for tissue engineering, so it is necessary to explore the molecular mechanism for their potential of multi-directional differentiation.

The PI3K/AKT signaling pathway is one of the crucial pathways in human body. This pathway consists of PI3K, AKT and downstream target proteins, participating in cell proliferation, differentiation, metabolism and apoptosis. The PI3K/AKT pathway can promote the proliferation of mouse and human embryonic stem cells, inhibit their differentiation, and essentially maintain the self-renewal ability5. PI3K is a specific kinase that catalyzes the hydroxyl phosphorylation of phosphatidylinositol to generate a second messenger. Until now, type I PI3K has been most widely studied6. PI3K/AKT is separated from the regulatory subunit p85 under the stimulation of tyrosine receptor kinase, G-protein-coupled receptor, Ras or other linker proteins, which gathers on the inner surface of cell membrane to form a special structure with kinase activity, then activating downstream signal proteins and participating in a variety of cellular activities7. The PI3K signaling pathway is involved in the induction of osteogenic differentiation of bone marrow mesenchymal stem cells by insulin-like growth factor-18.

As a specific ATP competitive inhibitor for type I PI3K discovered by Wang et al, LY294002 can specifically inhibit the catalytic activity of PI3Kp110 subunit, thereby blocking the downstream signaling pathway9. LY294002 can induce human embryonic stem cells to differentiate into insulin-secreting cells in vitro, which are more mature than insulin-secreting cells obtained from exendin-410. Besides, LY294002 can inhibit the osteogenic differentiation of dental pulp cells induced by lipopolysaccharide, suggesting that the PI3K signaling pathway may be involved11. The PI3K/AKT signaling pathway is widely involved in various activities of cells, but its role in DPSCs remains elusive. In this study, LY294002 was used to block the activity of the PI3K/AKT pathway in dental pulp cells. The effects of this signal pathway on the proliferation, migration and odontogenic differentiation of human DPSCs (hDPSCs) were evaluated, aiming to provide a theoretical basis for the repair and regeneration of dental pulp tissues.

Materials and Methods

Main reagents and apparatus

α-MEM (catalog number: 12571048), collagen type I (catalog number: 17018029), dispase (catalog number: 17105041) and trypsin (catalog number: 27250018) were purchased from Gibco (Waltham, USA). Fetal bovine serum (FBS) was bought from HyClone (catalog number: 10882-020).
Detection of hDPSC proliferation by CCK8 assay

After another 2 h of culture, the optical density (OD) at 450 nm was measured on a microplate reader, which was repeated 3 times. The normal pulp cells without inhibitor treatment were set as the control group.

Measurement of AKT, p-AKT and DSPP protein levels by Western blot

Detection of hDPSC migration by scratch assay

P3-generation hDPSCs were cultured in 10 cm petri dishes with serum-free α-MEM for 24 h when 70% confluence was reached. The cell surface was scratched along the midline of the petri dish with a 1 ml sterile pipette tip. Then the cell residue was washed away gently with PBS, and 10 μmol/l LY229402 was added. Photos were taken immediately under the inverted microscope (magnification: × 40) to record the distance between scratches at 0 h. The cells were thereafter incubated in a 5% CO₂ incubator at 37°C for 24 h, and paragraphed under the microscope (with the same visual field as that at 0 h) to record the scratches. The normal pulp cells without inhibitor treatment were set as the control group.
Figure 2. Effects of PI3K/AKT pathway on hDPSC migration. Scale bar = 50 μm.

Figure 3. Formation of mineralized nodules observed by alizarin red staining. A: Staining results observed by naked eyes and microscopy. Scale bar = 100 μm; B: quantitative analysis of staining results. *Compared with control group, P<0.05; #compared with mineralization induction group, P<0.05.
with the t test. P<0.05 was considered statistically significant.

Results

Effects of PI3K/AKT pathway on hDPSC proliferation

At 24, 48 and 72 h, the OD values of the LY group were significantly lower than those of the control group (P<0.05) (Fig. 1). Therefore, LY294002 evidently suppressed the proliferation of hDPSCs.

Effects of PI3K/AKT pathway on hDPSC migration

Compared with 0 h after scratching, the cells in each group migrated at 24 h. Compared with the control group, the number of migrating cells in the LY group was significantly lower. Thus, LY294002 markedly inhibited the migration of hDPSCs (Fig. 2).

Formation of mineralized nodules

After 14 days of mineralization induction, the mineralization induction group had most mineralized nodules, with the highest density (Fig. 3).

Effects of PI3K/AKT pathway on orthodontic/osteogenic differentiation of hDPSCs

The protein levels of p-AKT and AKT remained almost unchanged at 0, 6, 12, 24, 48 and 72 h. After addition of LY294002, the protein level of p-AKT decreased from 6 to 72 h, that of AKT did not change significantly, and that of odontogenic differentiation marker DSPP reduced (Fig. 4 and Table 1).

Discussion

PI3K is a family of lipid kinases that can be activated by a variety of cytokines and physicochemical factors. By catalyzing the phosphorylation of phosphatidylinositol (4,5) P2 to phosphoinositide (3,4,5) P3, it activates the downstream molecule AKT, causes the phosphorylation of mTOR, GSK-3β and NF-κB, activates or inhibits related target proteins, thereby regulating the biological processes such as transcription, translation, cell cycle, and apoptosis. PI3K activates this pathway by activating the downstream key protein AKT, which is a serine/threonine kinase. AKT is divided into three subtypes: AKT1, AKT2 and AKT3, which are composed of N-terminal PH domain, central domain and C-terminal kinase catalytic domain. Phosphatidylinositol 3,4,5-inositol triphosphate binds AKT and PDK1 at the N-terminal PH domain, and phosphorylation is located in the two sites of Thr-308 and C-terminal Ser-473 in the central region, which can activate AKT and play a biological effect. The level of AKT phosphorylation (i.e. p-AKT) can reflect the activation status of the PI3K pathway. In this study, the protein levels of AKT and p-AKT did not change significantly in normal hDPSCs cultured for 0-72 h, indicating that PI3K pathway remained relatively stable during the normal growth and differentiation of hDPSCs. After addition of inhibitor LY294002, the total AKT protein level did not change significantly, and the p-AKT level decreased, suggesting that the PI3K/AKT pathway was inhibited, and the activation status of the pathway was not related to the total AKT protein level.

The PI3K/AKT signaling pathway is involved in regulating the proliferation of multiple cells. The PI3K/AKT pathway can promote the proliferation of hepatic astrocytes, and promote the hypoxia-mediated proliferation and migration of mouse embryonic stem cells. As an upstream negative regulatory factor of PI3K, PTEN can inhibit the proliferation of malignant glioma by inhibiting the signaling pathway. In this study, LY294002 specifically inhibited the PI3K/AKT signaling pathway and the CCK8 method was used to detect the proliferation of hDPSCs. The results showed that LY294002 reduced the proliferation rate of the cells for 0-72 h, and the longer the action time was, the more obvious the inhibition effect was. Hence, the PI3K/AKT signaling pathway could affect the proliferation of hDPSCs and inhibit the activity of PI3K/AKT pathway. The results of cell scratch assay showed that compared with 0 h after scratch, the cells in each group migrated to the inner side of the trace 24 h after scratch, indicating that hDPSCs had the ability of migration; compared with the control group, the LY group had less cell migration. The number of cells in the LY group was significantly lower than that in the control group, suggesting that LY294002 inhibited the migration of hDPSCs. The PI3K/AKT signaling pathway is involved
in regulating the differentiation and self-renewal of various cells. Studies have confirmed that the PI3K/AKT pathway can inhibit cell senescence by promoting the self-renewal of skin progenitor cells. Lee et al. also confirmed that the PI3K/AKT signaling pathway promoted the osteogenic differentiation of bone marrow mesenchymal stem cells under hypoxic conditions, and regulated the osteogenic differentiation of human embryonic stem cells by modulating transcription factor Nanog. It has also reported to up-regulate bone differentiation markers BMP-2 and Runx2. There are a few hDPSCs in the pulp tissue. After external mechanical, chemical or bacterial stimulation, the odontoblasts located around the dental pulp and connected with the prephase dentin undergo degeneration, while hDPSCs differentiate into odontoblasts, forming the restorative dentin, protecting the pulp, and playing the defensive function. Herein, LY294002 inhibited the PI3K/AKT signaling pathway and examined the role of the PI3K/AKT pathway in the differentiation of dental pulp cells into dentin. Alizarin red staining was used to observe the formation of mineralized nodules. There were fewer mineralized nodules in the LY294002 group. After the treatment of LY294002 in the cells induced by mineralization, the number of mineralized nodules was significantly fewer than that in the purely mineralized group, which indicated that PI3K/AKT pathway affected the formation of mineralized nodules, and suggested that the PI3K/AKT signaling pathway might be involved in regulating the differentiation of dental pulp cells into dentin, and the inhibition of the PI3K pathway could reduce the formation of mineralized nodules. To further confirm the effect of PI3K/AKT pathway on the differentiation of hDPSCs into dentin, the protein level of DSPP was detected. DSPP is the main non-collagenous protein of teeth, which plays an important role in the mineralization of teeth and is a common sign of the odontogenic differentiation.

In summary, inhibition of the activity of the PI3K/AKT signaling pathway can reduce the proliferation and migration rates of hDPSCs, the formation of mineralized nodules, and the protein expression level of the dentin differentiation marker DSPP, which indicates that inhibition of the PI3K/AKT signaling pathway can inhibit the proliferation, migration and odontoblast differentiation of hDPSCs, and suggests that this signal pathway may play a role in the repair process of hDPSCs, whose specific mechanism needs to be further studied.

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Competing Interests

The authors declare that they have no competing interests.

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