Int J Clin Exp Med 2017;10(6):9171-9179
www.ijcem.com /ISSN:1940-5901/IJCEM0047185

Original Article

Periostin inhibits hypoxia-induced apoptosisin human periodontal ligament fibroblasts via the Akt/PKB signaling pathway

Hui-Li Liu*, Zhuo Chen*

*Department of Stomatology, Zhengzhou Central Hospital Affiliated to Zhengzhou University, Zhengzhou 450007, P. R. China. *Equal contributors.*

Received May 31, 2016; Accepted April 11, 2017; Epub June 15, 2017; Published June 30, 2017

Abstract: Periostin, an extracellular matrix protein, is mainly expressed in periodontal ligament fibroblasts, and it has been shown to inhibit hypoxia-induced apoptosis. However, the underling molecular mechanism of periostin-mediated regulation of apoptosis is not well known. The apoptosis rate of periodontal ligament fibroblasts was increased under hypoxic conditions. The protein expression levels of (HIF-1α) and (BNIP3) were upregulated in periodontal ligament fibroblasts under hypoxic conditions. Overexpression of periostin decreased hypoxia-induced apoptosis, as well as HIF-1α and BNIP3 protein level. The phosphorylation level of Akt/PKB on Ser473 was increased in periodontal ligament fibroblasts transfected with periostin. Periostin activated the Akt/PKB signaling pathway through the $\alpha_v\beta_3$ integrins pathway. The addition of an Akt/PKB inhibitor resulted in an increase in hypoxia-induced apoptosis compared with the control group. Moreover, the protective effect of periostin was disrupted in periodontal ligament fibroblasts co-treated with Akt/PKB inhibitor. Under hypoxic conditions, periostin decreases HIF-1α and BNIP3 expression levels, and inhibits apoptosis in human periodontal ligament fibroblasts via activating the Akt/PKB kinase signaling, which provides a novel insight into the regulation of apoptosis in periodontal diseases.

Keywords: Periostin, hypoxia, apoptosis, periodontal ligament, Akt/PKB

Introduction

Periodontal disease is classified as a highly prevalent oral inflammatory disease characterized by the progressive inflammatory destruction of the soft and hard tissues of the teeth. Several systemic and local risk factors have been proved to contribute to the initiation and progression of periodontitis. Occlusal trauma is an injury of the supportive periodontium and has been implicated in inflammatory alveolar bone resorption [1]. The physiological blood flow in human periodontal ligament was lower than that in pulpal or brain because occlusal forces was able to easily reduce blood flow in the periodontal ligament [2]. Compression or tension of the periodontal ligament leads to a reduction of blood flow and thereby causes ischaemia and local hypoxia [3, 4]. Hypoxia caused by occlusal trauma was related to apoptosis in periodontal ligament cells [5]. $CoCl_2$-mimic hypoxic treatment accelerated apoptosis in human periodontal ligament cells by promoting expression of hypoxia-inducible factor-1α (HIF-α) [6]. Therefore, hypoxia-induced apoptosis in human periodontal ligament fibroblasts is closely associated with periodontitis.

HIF-1α, a heterodimeric transcriptional factor, plays a vital role in maintaining oxygen (O_2) homeostasis in the cellular environment [7]. HIF-1α has been demonstrated to regulate the expression of numerous target genes that are involved in cancer biology, including angiogenesis, glucose metabolism, cell invasion and migration, and apoptosis [8]. Under normoxic conditions, HIF-1α was hydroxylated by prolyl-hydroxylases (PHDs) and ubiquitinated by the protein encoded by von Hippel-Lindau (Pvhl), leading to a rapid proteasomal degradation of HIF-1α. Under hypoxic conditions, the degradation pathway was blocked due to inactivation of PHDs [9, 10]. $CoCl_2$ has been used as a hypoxic mimicking agent. $CoCl_2$ upregulated HIF-1α

expression and interfered the binding of HIF-1α and PvhI, thus maintaining the stability of HIF-1α [11]. Bcl-2/E1B 19 kDa interacting protein 3 (BNIP3) has been shown to result in mitochondrial dysfunction and cell death in different types of cells [12]. The expression of BNIP3 was upregulated under hypoxic conditions. Inhibition of BNIP3 expression decreased hypoxia-induced apoptosis and myeloid cell leukemia 1 (MCL1) effectively abolished the ability of BNIP3 to induce cell death in malignant brain tumor glioblastoma under hypoxic conditions [13].

Periostin has been found abundantly expressed in fibrous connective tissues, including heart valves, tendons, perichondrium, and periodontal ligament [14]. Periostin promoted proliferation of differentiated cardiomyocytes and contributes to ventricular remodeling and angiogenesis [15]. In human gastric cancer SGC-7901 cells treated with cisplatin or 5-fluorouracil, overexpression of periostin decreased the expression levels of Bax and p53 proteins and increased the expression level of Bcl-2 protein. Moreover, upregulation of periostin inhibited cisplatin or 5-fluorouracil-induced apoptosis in SGC-7901 cells via the Akt/p53 signaling pathway [16]. Although a previous study has shown that periostin inhibited hypoxia-induced apoptosis in human periodontal ligament cells through modulating the TGF-β1/SMAD2 signaling pathway [17], the exact mechanism underlying the anti-apoptosis role of periostin still needs further research.

In the present study, the effect of periostin on human periodontal ligament fibroblasts apoptosis was elucidated under hypoxic conditions. The results showed that periostin exerted an antagonistic activity against the hypoxia-induced apoptosis in periodontal ligament fibroblasts and may serve as a specific target for therapy of periodontal diseases.

Materials and methods

Cell culture

Human periodontal ligament fibroblasts (Lonza, Walkersville, MD, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA) containing 100 U/ml penicillin (Sigma-Aldrich, Louis, MO, USA), 100 μg/ml streptomycin (Sigma-Aldrich), and 10% (vol/vol) heat-inactivated fetal bovine serum (Gibco) at 37°C in a humid incubator with 5% CO_2.

Plasmids and cell treatment

For construction of a periostin-expressing plasmid [14], full-length human periostin cDNA (GenBank NM_006475) was amplified by PCR with primers: forward, 5'-GCCGAAGCTTACCATGATTCCC-3'; reverse, 5'-GCGCCTCGAGTCACTGAGAACG-3'. The fragment was digested with H*ind* III and X*ho* I and subcloned into pcDNA3.1(+) vector (Invitrogen). The constructed plasmid (pcDNA3.1-periostin) was confirmed by DNA sequencing. Cells were firstly seeded onto a 6-well plate (1×10^6 cells/well). After incubation for 24 h, cells were transfected with pcDNA3.1(+) empty vector (0.5 μg), pcDNA3.1/periostin plasmid (0.5 μg) using lipofectamine-2000 reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. For inhibition of the Akt/PKB signaling pathway, human periodontal ligament fibroblasts were treated with 15 mM of 1L-6-Hydroxymethyl-chiroinositol 2-®-2-O-methyl-3-O-octadecylcarbonate (Merck Biosciences, Nottingham, UK) for 45 min.

Induction of hypoxic condition

Cells were transferred to an airtight chamber containing AnaeroPack system combined with AnaeroPack oxygen indicator (Mitsubishi Gas Chemical Co. Inc., Tokyo, Japan). The agent can absorb O_2 entirely within 0.5-1 hour. The hypoxic conditions were obtained by using Anaeropack System (Mitsubishi Gas Chemical Co. Inc., Tokyo, Japan). We used different amount of the agent to keep O_2 concentration at approximate 1% and the concentration of O_2 was indicated by AnaeroPack oxygen indicator showing different colors.

Analysis of apoptosis by flow cytometry

An annexin V-fluorescein isothiocyanate (FITC) apoptosis detection Kit (BD Biosciences Pharmingen, San Jose, CA, USA) was used according to the manufacturer's instructions. The cells were harvested and washed twice with cold phosphate-buffered saline (PBS) and then resuspended in cold 1 × binding buffer. Annexin V-FITC (5 μl) and propidium iodide (PI, 5 μl) were then added to the cell suspensions and then

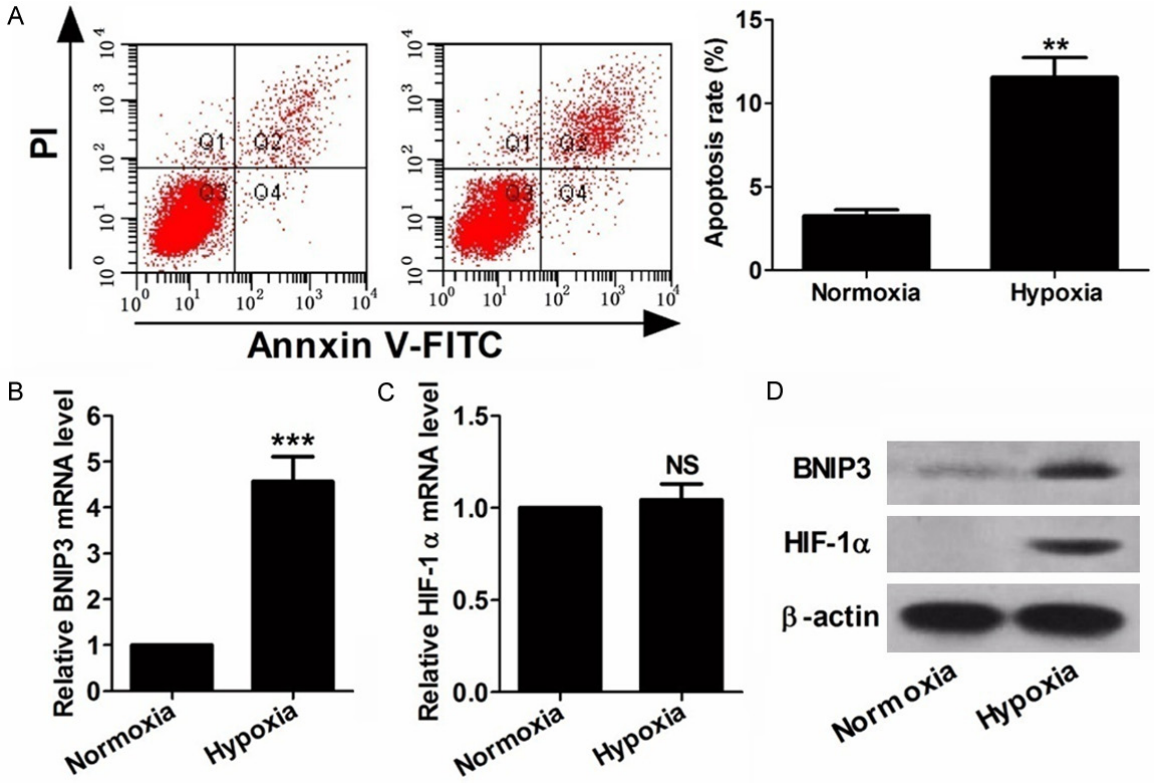

Figure 1. Hypoxia promotes apoptosis and regulates HIF-1α and BNIP3 expression in human periodontal ligament fibroblasts. (A) Flow cytometry analysis was performed by annexin-V-FITC/PI dual staining. The lower right quadrant of each panel represents the percentage of early apoptotic cells (annexin V^+ PI^-) and the upper right quadrant indicates the percentage of late apoptotic cells (annexin V^+ PI^+). After 48 h of hypoxia, the expression of HIF-1α and BNIP3 were assessed by qRT-PCR (B and C) and western blot (D). Data were represented as the mean ± SD (n = 3). $^{**}P < 0.01$; $^{***}P < 0.001$; NS, not significant.

incubated at room temperature for 15 min in a dark place. Stained cells were analyzed on a FACScan flow cytometer (Becton Dickinson, CA, USA) using Cellquest software (Becton Dickinson).

Western blot

At 48 h after transfection, cells were washed twice with PBS and then total proteins were extracted with RIPA lysis buffer (Beyotime Institute of Biotechnology, Jiangsu, China). The protein concentration was determined using bicinchoninic acid reagent (Beyotime Institute of Biotechnology). Equal amount of protein samples was loaded into the individual wells and separated by polyacrylamide gel electrophoresis, and then transferred to polyvinylidene fluoride membranes. Membranes were blocked with 5% non-fat milk powder in Tris buffered saline containing 0.1% Tween-20 (TBST) for 1 h at room temperature and then incubated with the anti-periostin, anti-BNIP3, anti-HIF-1α, anti-$pAkt_{ser473}$, anti-Akt or anti-β-actin antibody at 4°C overnight. β-actin was used as a loading control. The membranes were washed with TBST (3 × 10 min) and then incubated with the corresponding horseradish-peroxidase-labeled secondary antibody for 1 h at room temperature. Following washes with TBST (3 × 15 min), the blots were visualized using enhanced chemi-luminescence detection kit (Pierce, Rockford, IL, USA). The band density was measured using the Image J software (National Institutes of Health, Bethesda, MD, USA).

Real-time RT-PCR

Total RNA was isolated from the cells with TRIzol reagent (Invitrogen) and purified using a RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. The RNA concentration was determined using an Agilent Bioana-

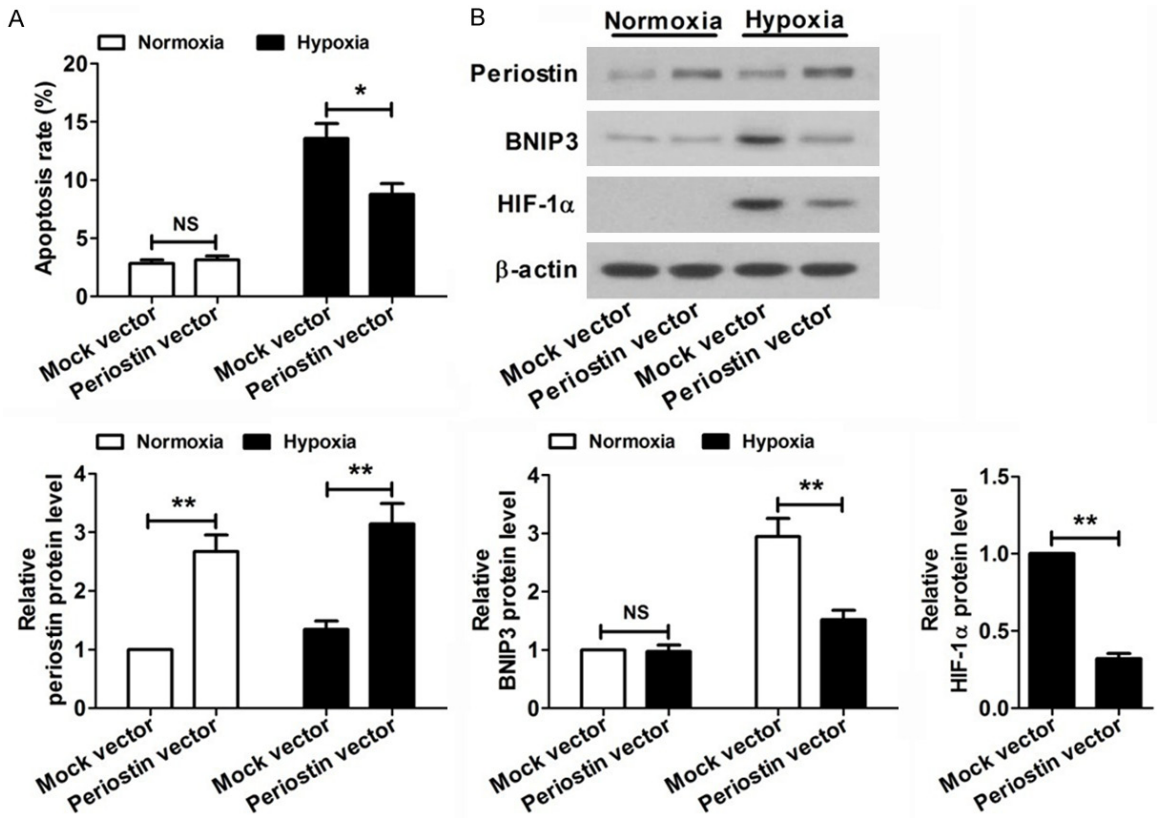

Figure 2. Periostin alleviates hypoxia-induced apoptosis and upregulation of HIF-1α and BNIP3 expression. After transfection with empty vector (mock vector) or periostin vector, human periodontal ligament fibroblasts were subjected to hypoxia (1% O_2) treatment for 48 h. A. Cells were stained with annexin V-FITC and PI, and then analyzed by flow cytometry analysis. B. Cells were subjected to western blot analysis. Data were represented as the mean ± SD (n = 3). *P < 0.05; **P < 0.01; NS, not significant.

lyzer 2000 RNA Nanochips (Agilent, Santa Clara, CA, USA). A total of 150 ng of RNA template was reversely transcribed into complementary DNA (cDNA) using TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, USA). qRT-PCR was performed on an ABI 7900 qRT-PCR system (Thermo Scientific-Applied Biosystems, Foster City, CA, USA) using Fast SYBR Green master mix (Applied Biosystems) in accordance with the manufacturer's protocol. The expression level of BNIP3 and HIF-1α mRNA were normalized to β-actin expression and calculated according to the $2^{-\Delta\Delta Ct}$ method.

Statistical analyses

Student's *t*-test or analysis of variance was conducted to determine statistically significant differences using the SPSS software package for windows (version17.0; SPSS Inc., Chicago, IL, USA). A *P* value of less than 0.05 was considered to be significant for these analyses. Each experiment was repeated at least three times.

Results

Hypoxia induced apoptosis and affected HIF-1α and BNIP3 expression

To assess the effect of hypoxia on survival of periodontal ligament fibroblasts, flow cytometry was used to detect apoptotic cells. Representative dot plots of annexin-V-FITC/PI dual staining were shown in **Figure 1A**. After 48 h of hypoxia, the apoptosis rate of periodontal ligament fibroblasts was significantly increased compared with the control group. The mRNA expression level of BNIP3 in the hypoxia group was higherthan the normoxia group (**Figure 1B**). However, no change in HIF-1α mRNA level was observed in response to hypoxia (**Figure 1C**). Furthermore, protein expression of HIF-1α and BNIP3 were both enhanced in the hypoxia group (**Figure 1D**).

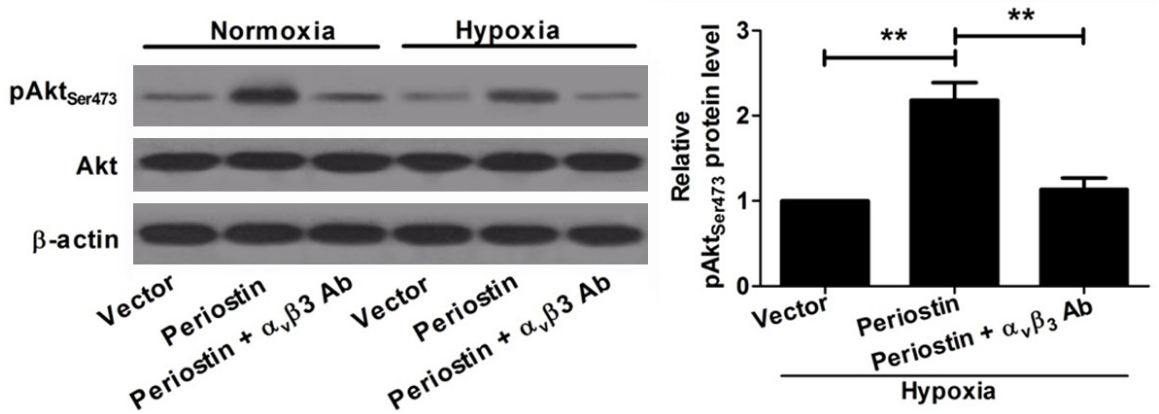

Figure 3. Periostin evokes the Akt/PKB pathway under normoxic and hypoxic conditions. Cells were transfected with periostin vector or empty vector or treated with periostin + $\alpha_v\beta_3$Ab, then cells were subjected to hypoxia (1% O_2) treatment for 48 h. The phosphorylation level of Akt on Ser473 was detected using western blot under normoxic and hypoxic conditions, respectively. Data were represented as the mean ± SD (n = 3). $^{**}P < 0.01$.

Periostin inhibited apoptosis and decreased HIF-1α and BNIP3 expression under hypoxic conditions

To investigate the roles of periostin in the process of apoptosis induced by hypoxia, human periodontal ligament fibroblasts were transfected with periostin expression plasmids or empty vector. The effect of periostin on hypoxia-induced apoptosis was evaluated by flow cytometry. The results showed that apoptosis rate of periodontal ligament fibroblasts was decreased in the periostin group compared with the control group (**Figure 2A**), suggesting that periostin promoted cellular survival under hypoxic conditions. Furthermore, the protein levels of periostin, HIF-1α and BNIP3 were measured by western blot analysis. Under hypoxic conditions, the protein expression levels of both HIF-1α and BNIP3 were decreased in the periostin group when compared with the mock vector group (**Figure 2B**). However, under normoxic condition, there was no obvious difference in BNIP3 and HIF-1α protein level between periostin group and control group (**Figure 2B**).

Periostin activated the Akt/PKB pathway in human periodontal ligament fibroblasts

Apoptosis of periodontal ligament fibroblasts has been shown to be involved in the pathogenesis of periodontal diseases. To make clear the molecular mechanism by which periostin inhibits hypoxia-induced apoptosis, The activity of Akt/PKB cell survival signaling pathway was examined. Transfection of periostin drastically increased the phosphorylation level of Akt/PKB on Ser473 (pAkt$_{Ser473}$) in periodontal ligament fibroblasts, however, this effect was abrogated by the $\alpha_v\beta_3$ integrins antibody ($\alpha_v\beta_3$Ab) (**Figure 3**). These results indicated that periostin activated Akt/PKB kinase signaling via the $\alpha_v\beta_3$ integrins signaling pathway.

Inhibition of the Akt/PKB pathway disruptedthe inhibitory ability of periostin against hypoxia-induced apoptosis

To further confirm that periostin inhibits hypoxia-induced apoptosis by activating the Akt/PKB signaling pathway, a specific inhibitor of Akt/PKB kinase, 1L-6-Hydroxymethyl-chiroinositol 2-(R)-2-O-methyl-3-O-octadecylcarbonate, was used to block the Akt/PKB kinase activity. pcDNA-periostin transfected cells or empty vector transfected cells were treated with vehicle (DMSO) or Akt/PKB inhibitor (15 μM) for 45 min, then cells were subjected to hypoxia (1% O_2) treatment for 48 h. Western blot analysis was used to detect related protein expression. As shown in **Figure 4A**, transfection of periostin evidently increased periostin protein expression, and Akt/PKB inhibitor had no effect on periostin expression. Additionally, periostin dramatically suppressed HIF-1α expression, and Akt/PKB inhibitor notably promoted HIF-1α expression; while Akt/PKB inhibitor abated the inhibitory effect of periostin on HIF-1α level under hypoxic condition. Moreover, periostin

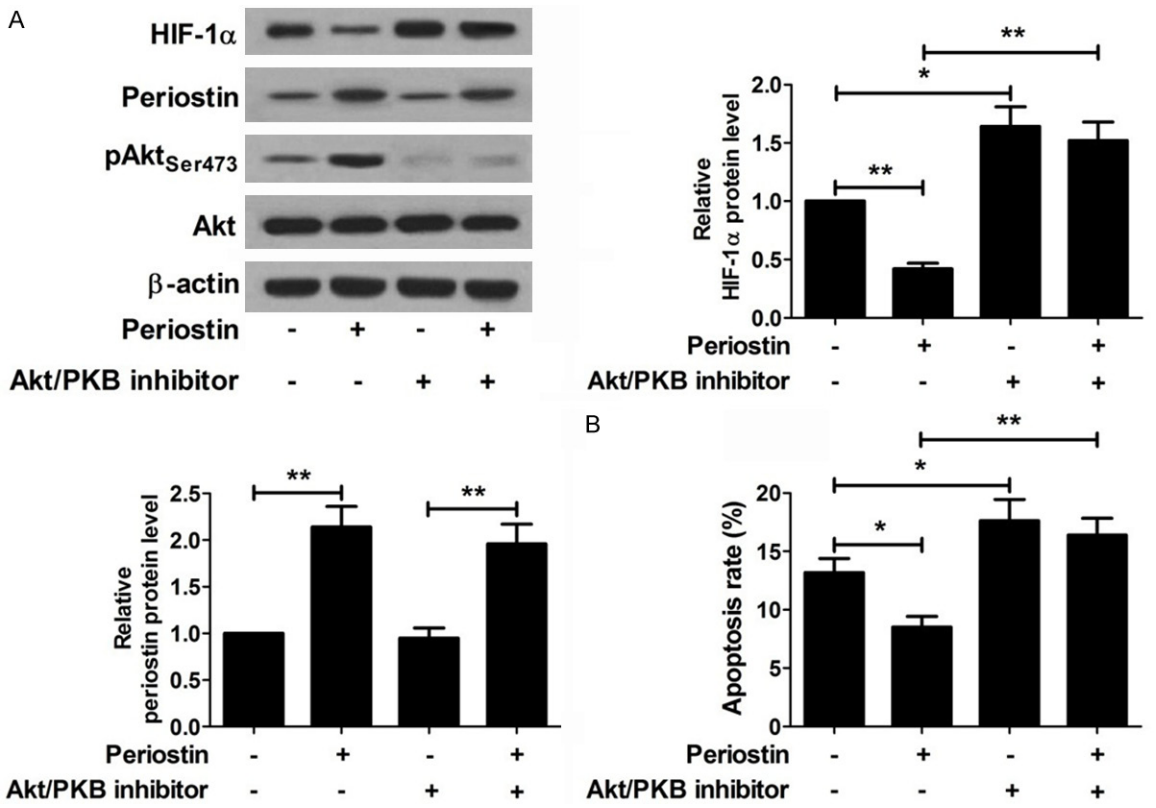

Figure 4. The Akt/PKB inhibitor decreases the inhibitory effect of periostin against hypoxia-induced apoptosis. pcDNA-periostin transfected cells or empty vector transfected cells were treated with vehicle (DMSO) or Akt/PKB inhibitor (15 μM) for 45 min, then cells were subjected to hypoxia (1% O_2) treatment for 48 h. A. Western blot was performed to detect the protein expression levels of HIF-1α, periostin, pAkt$_{Ser473}$ and Akt in periodontal ligament fibroblasts under different conditions. B. The amount of apoptotic cells was identified by flow cytometry. Data were represented as the mean ± SD (n = 3). *P < 0.05; **P < 0.01.

dramatically elevated pAkt$_{Ser473}$ level, and Akt/PKB inhibitor decreased pAkt$_{Ser473}$ level; while Akt/PKB inhibitor overturned the positive effect of periostin on pAkt$_{Ser473}$ expression under hypoxic condition. In addition, the apoptotic cells were detected by flow cytometry. As shown in **Figure 4B**, transfection of periostin resulted in a decrease in apoptosis rate of periodontal ligament fibroblasts, and treatment of Akt/PKB inhibitor led to an increase in apoptosis rate. In addition, Akt/PKB inhibitor significantly abolished the inhibitory effect of periostin on cell apoptosis under hypoxic condition. These data suggested that periostin inhibits hypoxia-induced apoptosis in human periodontal ligament fibroblasts via the Akt/PKB signaling pathway.

Discussion

The injury of periodontal tissues, including periodontal ligament and cementum, is a main cause of tooth loss in adults and is a severe public-health burden around the world. Excessive apoptosis of periodontal ligament fibroblasts plays a vital role in the progression of periodontitis [18]. Here, the role of periostin in apoptosis in human periodontal ligament fibroblasts was investigated under hypoxic conditions. The results suggested that periostin inhibited hypoxia-induced apoptosis in human periodontal ligament fibroblasts through modulating the Akt/PKB signaling pathway. Our study identified an important mechanism underlying the inhibition of hypoxia-induced apoptosis.

Apoptosis is a genetically encoded programme of cell death and is triggered by various cellular stresses. Abnormal apoptosishas been implicated in many human diseases, including neurodegenerative diseases, cardiovascular disease, ischemic damage, periodontal diseases and a variety of cancers [19]. A number of apoptotic factors, including Caspase-3, Smac

and Bax, have been demonstrated to be associated with periodontal tissue destruction [20]. The apoptosis is activated in response to multiple stressors, including UV radiation, hypoxia, mechanical injury, oxidative stress and endoplasmic reticulum stress. The exact mechanisms underlying hypoxia-induced cell death are controversial. Different types of studies have shown that apoptosis, necrosis and autophagy are induced by hypoxic stress. A previous study reported that prolonged hypoxia induced autophagic cell death in five different cell lines, which was accompanied with a upregulation of the pro-cell-death Bcl-2 family member BNIP3 [21]. Zhu *et al.* have demonstrated that hypoxia induces cytochrome C release, Bax accumulation and activation of caspase-3, resulting in apoptosis of rat mesenchymal stem cells [22].

O_2 is required for aerobic organisms to produce energy and acts as an electron acceptor in numerous organic and inorganic reactions. O_2 deprivation causes additional stress on proteins and DNA and thereby reduces the growth and viability of living cells. Multiple signaling pathways, including the unfolded protein response (UPR), mTOR signaling, Notch signaling and HIFs, are involved in hypoxia-induced pathological processes [23]. Hypoxia results in accumulation of HIF-1α and HIF-1β, and affects the expression of proteins which are involved in cell development, metabolism, proliferation and apoptosis [24]. Apoptosis is induced in response to severe hypoxia. Hypoxia or $CoCl_2$ treatment has been shown to be correlated with pro-apoptotic and anti-apoptotic proteins. $CoCl_2$ reduces the expression of proteasome subunit β5, low molecular mass polypeptide-7 (LMP7) and LMP2, as well as the activity of proteasome and immunoproteasome [25]. HIF-1 could initiate hypoxia mediated apoptosis by upregulating the expression of pro-apoptotic protein BNIP3 and stabilizing p53 [26]. The expression levels of HIF-1α and BNIP3 protein and the expression level of BNIP3 mRNA were upregulated in human periodontal ligament cells with hypoxic treatment [27]. In accordance with the previous study [28], we found that hypoxia induced upregulation of HIF-1α expression at protein level, but the expression level of HIF-1α mRNA was not changed. Furthermore, hypoxia contributed to cell apoptosis in periodontal ligament fibroblasts.

The serine/threonine kinase Akt/PKB signaling is frequently dysregulated in a variety of human diseases, suggesting that the Akt-dependent pathways may be an effective target for therapeutic intervention. Akt also has been proved as an essential substrate for phosphorylation, acetylation, and ubiquitination in different kinds of cells. A cyclooxygenase-2 inhibitor SC236 induces apoptosis of gastric cancer cell lines via downregulation of Akt and upregulation of cytochrome c [29]. Liver X receptors induced apoptosis at least partially through inhibiting activity of the AKT survival pathway in prostate cancer cells [30]. Bao *et al.* demonstrated that periostin protected cells against stress-induced death by activating the Akt/PKB signaling pathway [31]. The activation of $\alpha_V\beta_3$ integrin has been proved to be involved in the regulation of the Akt signaling pathway [32]. Suppression of $\alpha_V\beta_3$ integrin by a functional blocking antibody inhibits the PI3K/AKT signaling pathway and the expression of vascular endothelial growth factor (VEGF), indicating that $\alpha_V\beta_3$ integrin is a key factor for PI3K/AKT-mediated VEGF expression [33]. Here, this study showed that periostin activated the Akt/PKB signaling pathway through the $\alpha_v\beta_3$ integrins pathway. Moreover, inactivation of Akt/PKB kinase by a specific inhibitor disrupts the inhibitory effect of periostin on hypoxia-induced apoptosis in human periodontal ligament fibroblasts.

In conclusion, these data demonstrate that periostin attenuates hypoxia-induced the HIF-1α protein expression in human periodontal ligament fibroblasts. Importantly, this study reveals that periostin inhibits hypoxia-induced apoptosis in human periodontal ligament fibroblasts by activating the Akt/PKB signaling pathway. This study identifies the molecular mechanism underlying the involvement of periostin in the regulation of apoptosis in human periodontal ligament fibroblasts under hypoxic conditions.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure of conflict of interest

None.

Address correspondence to: Zhuo Chen, Department of Stomatology, Zhengzhou Central Hospital Affiliated to Zhengzhou University, 195 Tongbai Road, Zhengzhou 450007, P. R. China. E-mail: calmzchen@163.com

References

[1] Caviedes-Bucheli J, Azuero-Holguin MM, Correa-Ortiz JA, Aguilar-Mora MV, Pedroza-Flores JD, Ulate E, Lombana N and Munoz HR. Effect of experimentally induced occlusal trauma on substance P expression in human dental pulp and periodontal ligament. J Endodont 2011; 37: 627-30.

[2] Tomokiyo A, Maeda H, Fujii S, Monnouchi S, Wada N, Kono K, Yamamoto N, Koori K, Teramatsu Y and Akamine A. A multipotent clonal human periodontal ligament cell line with neural crest cell phenotypes promotes neurocytic differentiation, migration, and survival. J Cell Physiol 2012; 227: 2040-50.

[3] Zainal Ariffin SH, Yamamoto Z, Megat Abdul Wahab R and Zainal Ariffin Z. Cellular and molecular changes in orthodontic tooth movement. ScientificWorldJournal 2011; 11: 1788-803.

[4] He Y, Macarak EJ, Korostoff JM and Howard PS. Compression and tension: differential effects on matrix accumulation by periodontal ligament fibroblasts in vitro. Connec Tissue Res 2004; 45: 28-39.

[5] Motohira H, Hayashi J, Tatsumi J, Tajima M, Sakagami H and Shin K. Hypoxia and reoxygenation augment bone-resorbing factor production from human periodontal ligament cells. J Periodontol 2007; 78: 1803-9.

[6] Yoon SY, Lee HR, Park Y, Kim JH, Kim SY, Yoon SR, Lee WJ, Cho BJ, Min H and Bang JW. Thymosin β4 expression correlates with lymph node metastasis through hypoxia inducible factor-α induction in breast cancer. Oncol Rep 2011; 25: 23-31.

[7] Papandreou I, Cairns RA, Fontana L, Lim AL and Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab 2006; 3: 187-97.

[8] Morfoisse F, Kuchnio A, Frainay C, Gomez-Brouchet A, Delisle MB, Marzi S, Helfer AC, Hantelys F, Pujol F and Guillermet-Guibert J. Hypoxia induces VEGF-C expression in metastatic tumor cells via a HIF-1α-independent translation-mediated mechanism. Cell Rep 2014; 6: 155-67.

[9] Jung JE, Kim HS, Lee CS, Shin YJ, Kim YN, Kang GH, Kim TY, Juhnn YS, Kim SJ and Park JW. STAT3 inhibits the degradation of HIF-1α by pVHL-mediated ubiquitination. Exp Mol Med 2008; 40: 479-85.

[10] Jiang X, Zhang D, Zhang H, Huang Y and Teng M. Corrigendum: role of Ran-regulated nuclear-cytoplasmic trafficking of pVHL in the regulation of microtubular stability-mediated HIF-1α in hypoxic cardiomyocytes. Sci Rep 2015; 5: 11307.

[11] Ardyanto TD, Osaki M, Tokuyasu N, Nagahama Y and Ito H. CoCl2-induced HIF-1α expression correlates with proliferation and apoptosis in MKN-1 cells: a possible role for the PI3K/Akt pathway. Int J Oncol 2006; 29: 549-555.

[12] Gonzalez ER, Hammerling B, Hanna R, Kubli DA and Gustafsson ÅB. The BH3-only protein BNIP3 induces mitochondrial clearance via multiple pathways. Circ Res 2015; 117: A174-4.

[13] Chen Y, Henson ES, Xiao W, Shome E, Azad MB, Burton TR, Queau M, Sathya A, Eisenstat DD and Gibson SB. Bcl-2 family member Mcl-1 expression is reduced under hypoxia by the E3 ligase FBW7 contributing to BNIP3 induced cell death in glioma cells. Cancer Biol Ther 2015; 17: 604-13.

[14] Norris RA, Damon B, Mironov V, Kasyanov V, Ramamurthi A, Moreno-Rodriguez R, Trusk T, Potts JD, Goodwin RL and Davis J. Periostin regulates collagen fibrillogenesis and the biomechanical properties of connective tissues. J Cell Biochem 2007; 101: 695-711.

[15] Kühn B, del Monte F, Hajjar RJ, Chang YS, Lebeche D, Arab S and Keating MT. Periostin induces proliferation of differentiated cardiomyocytes and promotes cardiac repair. Nat Med 2007; 13: 962-9.

[16] Li B, Wang L and Chi B. Upregulation of periostin prevents P53-mediated apoptosis in SGC-7901 gastric cancer cells. Mol Biol Rep 2013; 40: 1677-83.

[17] Aukkarasongsup P, Haruyama N, Matsumoto T, Shiga M and Moriyama K. Periostin inhibits hypoxia-induced apoptosis in human periodontal ligament cells via TGF-β signaling. Biochem Biophys Res Commun 2013; 441: 126-32.

[18] Gjertsen AW, Stothz KA, Neiva KG and Pileggi R. Effect of propolis on proliferation and apoptosis of periodontal ligament fibroblasts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112: 843-8.

[19] Tomokiyo A, Maeda H, Fujii S, Monnouchi S, Wada N, Hori K, Koori K, Yamamoto N, Teramatsu Y and Akamine A. Alternation of extracellular matrix remodeling and apoptosis by activation of the aryl hydrocarbon receptor pathway in human periodontal ligament cells. J Cell Biochem 2012; 113: 3093-103.

[20] Cheng R, Choudhury D, Liu C, Billet S, Hu T and Bhowmick N. Gingival fibroblasts resist apoptosis in response to oxidative stress in a model of periodontal diseases. Cell Death Dis 2015; 1: 15046.

[21] Azad MB, Chen Y, Henson ES, Cizeau J, McMillan-Ward E, Israels SJ and Gibson SB. Hypoxia induces autophagic cell death in apoptosis-competent cells through a mechanism involving BNIP3. Autophagy 2008; 4: 195-204.
[22] Zhu W, Chen J, Cong X, Hu S and Chen X. Hypoxia and serum deprivation-induced apoptosis in mesenchymal stem cells. Stem Cells 2006; 24: 416-25.
[23] Majmundar AJ, Wong WJ and Simon MC. Hypoxia-inducible factors and the response to hypoxic stress. Mol Cell 2010; 40: 294-309.
[24] Heyman SN, Rosen S and Rosenberger C. Hypoxia-inducible factors and the prevention of acute organ injury. Crit Care 2011; 15: 209.
[25] Eleftheriadis T, Pissas G, Antoniadi G, Liakopoulos V and Stefanidis I. Proteasome or immunoproteasome inhibitors cause apoptosis in human renal tubular epithelial cells under normoxic and hypoxic conditions. Int Urol Nephrol 2016; 48: 907-15.
[26] Greijer A and Van der Wall E. The role of hypoxia inducible factor 1 (HIF-1) in hypoxia induced apoptosis. J Clin Pathol 2004; 57: 1009-14.
[27] Song ZC, Zhou W, Shu R and Ni J. Hypoxia induces apoptosis and autophagic cell death in human periodontal ligament cells through HIF-1α pathway. Cell Prolif 2012; 45: 239-48.
[28] Wu H, Huang S, Chen Z, Liu W, Zhou X and Zhang D. Hypoxia-induced autophagy contributes to the invasion of salivary adenoid cystic carcinoma through the HIF-1α/BNIP3 signaling pathway. Mol Med Rep 2015; 12: 6467-74.
[29] Fan XM, Jiang XH, Gu Q, Ching YP, He H, Xia HH, Lin MC, Chan AO, Yuen MF and Kung HF. Inhibition of Akt/PKB by a COX-2 inhibitor induces apoptosis in gastric cancer cells. Digestion 2006; 73: 75-83.
[30] Pommier A, Alves G, Viennois E, Bernard S, Communal Y, Sion B, Marceau G, Damon C, Mouzat K and Caira F. Liver X receptor activation downregulates AKT survival signaling in lipid rafts and induces apoptosis of prostate cancer cells. Oncogene 2010; 29: 2712-23.
[31] Bao S, Ouyang G, Bai X, Huang Z, Ma C, Liu M, Shao R, Anderson RM, Rich JN and Wang XF. Periostin potently promotes metastatic growth of colon cancer by augmenting cell survival via the Akt/PKB pathway. Cancer Cell 2004; 5: 329-39.
[32] Cam A and de Mejia EG. RGD-peptide lunasin inhibits Akt-mediated NF-κB activation in human macrophages through interaction with the αVβ3 integrin. Mol Nutr Food Res 2012; 56: 1569-1581.
[33] Chetty C, Lakka SS, Bhoopathi P and Rao JS. MMP-2 alters VEGF expression via αVβ3 integrin-mediated PI3K/AKT signaling in A549 lung cancer cells. Int J Cancer 2010; 127: 1081-1095.