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Xeroderma Pigmentosum Group D (XPD) Inhibits the Proliferation Cycle of Vascular Smooth Muscle Cell (VSMC) by Activating Glycogen Synthase Kinase 3 β (GSK3 β)

Authors' Contribution:
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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: VSMC proliferation plays a key role in atherosclerosis, but the role of XPD in VSMC proliferation remains unknown. We investigated the expression of XPD, which is involved in cell cycle regulation, and its role in VSMC proliferation response to atherogenic stimuli.


Material/Methods: Human umbilical vein VSMCs were transfected with recombinant plasmid pEGFP-N2/XPD and pEGFP-N2 and incubated with PDGF-BB *in vitro*. Cell viability was determined by MTT assay. The expressions of XPD, GSK3 β , p-GSK3 β , CDK4, and cyclin D1 protein were detected by Western blot analysis. Cell cycle was examined by flow cytometry.

Results: PDGF inhibited the expression of XPD in VSMCs and promoted VSMC proliferation. Overexpression of XPD significantly augmented cell cycle arrest, and attenuated protein expression levels of CDK4 and cyclin D1 in VSMCs. XPD overexpression suppressed the effects of PDGF-BB in promoting G1/S transition and accelerating protein expression levels of CDK4 and cyclin D1. XPD diminished the phosphorylation of GSK3 β , and SB216763 inhibited the reduction effect of XPD on CDK4 and cyclin D1.

Conclusions: XPD induces VSMC cell cycle arrest, and the activation of GSK3 β plays a crucial role in inhibitory effect of XPD on VSMC proliferation.

MeSH Keywords: **Cell Cycle • Glycogen Synthase Kinase 3 • Muscle, Smooth, Vascular • Xeroderma Pigmentosum Group D Protein**

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Background

The generation of atherosclerosis results from complex interactions of atherogenic stimuli such as oxidized phospholipids, inflammatory factors, and growth factors, and various cell types in the vessel wall, including endothelial cells, lymphocytes, monocytes, and smooth muscle cells [1]. Recent studies revealed the important role of vascular smooth muscle cells (VSMCs) in the initiation and progression of atherosclerotic plaque [2,3]. At the early stage of atherosclerosis, VSMCs that have switched phenotype induced by atherogenic stimuli after vascular endothelial injury migrate into the neointima and secrete extracellular matrix, which is critically involved in neointima formation and development [4]. In addition, augmented VSMC proliferation plays a key role in intimal hyperplasia, a common pathological characteristic in atherosclerosis and restenosis after angioplasty [5]. Therefore, identifying the regulatory factors of VSMC proliferation may facilitate the development of therapies for preventing intimal thickening.

There is increasing evidence of DNA damage accumulation in the development of atherosclerosis. Atherogenic risk factors, such as age, smoking, hypertension, hypercholesterolemia, and diabetes, can induce DNA damage by action of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [6,7]. The DNA damage response (DDR) pathway can be activated to ensure genetic stability when DNA damage is detected. There are 4 main DDR pathways in mammals: nucleotide excision repair (NER), base excision repair (BER), double-strand break repair (DSB repair), and mismatch repair (MMR) [8,9].

Transcription factor II H (TFIIH) plays a crucial role in eukaryotic transcription initiation and in the NER pathway. It could be divided to 2 main groups: the core- TFIIH consists of XPB, p52, p8, p62, p44, and p34, and the cyclin-dependent kinase (CDK)-activating kinase (CAK) subunit complex contains CDK7, cyclin H, and MAT1 [10]. Xeroderma pigmentosum group D (XPD) is an important component of DNA repair factor (TFIIH), which bridges the 2 groups. Multiple studies have demonstrated that XPD can modulate cellular proliferation [11,12], while the effects of XPD in cultured VSMCs remain largely uncharacterized. The role of XPD in VSMC proliferation and whether XPD is affected in VSMCs treated with atherogenic stimuli remain unknown. Therefore, we investigate the role of XPD in proliferation of VSMCs induced by PDGF-BB *in vitro*.

Material and Methods

Cell culture

Human umbilical vein VSMCs (Guangzhou Jenniobio Biotechnology Co., Ltd.) were maintained in RPMI-1640 (Hyclone,

USA) supplemented with 12% FBS (Biological Industries, Israel) in a humidified incubator with 5% CO₂ at 37°C. Cells were subcultured when 80% synchronized and passages between 5 and 7 were seeded in 6-well plates, unless otherwise stressed, with an appropriate density.

Cell viability assay

Cell viability was determined by MTT assay. Human umbilical vein VSMCs were seeded in 96-well plates at a density of 5×10^4 cells/well. After PDGF-BB (RayBiotech, USA) incubation, the cells were washed twice with PBS, followed by incubation with 25 μ l of MTT (Sigma, USA) at 37°C for 4 h in the dark. Subsequently, 150 μ l of DMSO (Sigma, USA) was added to dissolve MTT crystals. The absorbance was measured in a microplate reader at 490 nm (Bio-Rad 680, USA).

Plasmid transfection

Plasmid was constructed by Jiangxi Provincial Key Laboratory of Molecular Medicine as described previously [12]. VSMCs were seeded in 6-well plates at a density of 5×10^4 cells/well in growth medium without antibiotics. When cells were attached to the wall, the medium was replaced by serum-free medium. One hour later, the cells were transfected with pEGFP-N2 or pEGFP-N2/XPD mixed with cationic lipid by Lipofectamine™ 2000 Transfection Reagent (Invitrogen) following the manufacturer's guidelines. The expression of GFP was observed under a fluorescence microscope (Nikon). The expression levels of XPD in the cells with or without XPD transfected were determined by Western blot analysis.

Cell cycle analysis

Cells were seeded in 25-cm² flasks and then pre-incubated in RPMI-1640 supplemented with 0.2% FBS for 24 h, which induced cell cycle synchronization. Cells were harvested using EDTA-free trypsin, washed twice with ice-cold PBS, fixed, and permeabilized with ice-cold 70% ethanol. The cells were treated with 50 μ g/ml PI in the dark at 4°C for 30 min. The processes cells were analyzed by a flow cytometer (FACSCalibur, Beckton Dickinson, USA).

Western blot analysis

After treatment, VSMCs were lysed in lysis buffer (62.5 mM Tris-HCl, pH 7.6, 2% SDS, 25% glycerol, 0.01% bromochlorophenol blue, 5% β -mercaptoethanol) on ice and centrifuged at 12 000 g for 15 min at 4°C. The lysates were resolved on SDS-PAGE (10% resolving, 5% stacking) before transfer onto polyvinylidene fluoride membranes (Millipore, USA). The membranes were blocked in 5% skim milk followed by incubation with primary antibodies (XPD 1: 1000, CDK4 1: 500, cyclin D1 1: 1000, p-GSK3 β

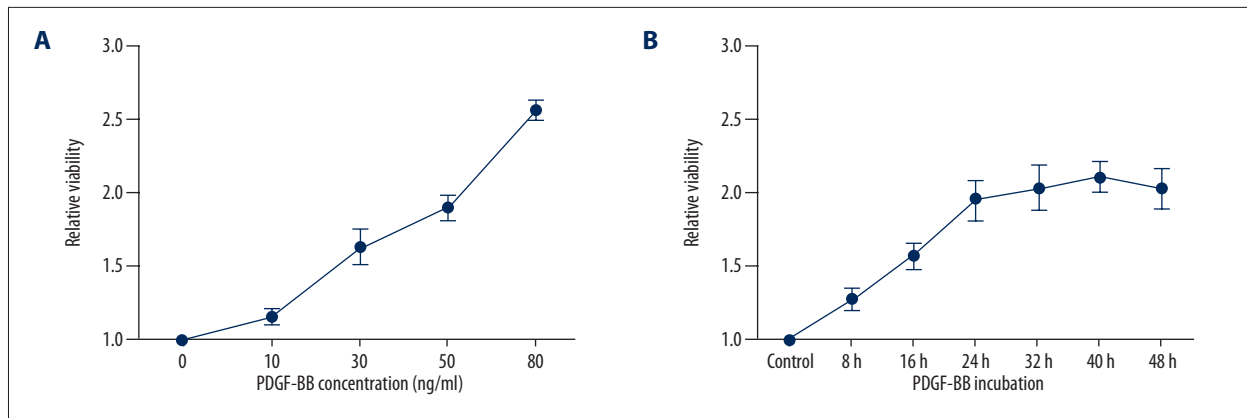


Figure 1. Cell viability was evaluated by MTT assay. (A) The cell viability increased in a dose-dependent manner within the concentration of PDGF-BB at 10 ng/ml, 30 ng/ml, and 80 ng/ml. (B) The cell viability increased in a time-dependent manner within 0 h, 8 h, 16 h, 24 h, 32 h, 40 h, and 48 h of incubation time.

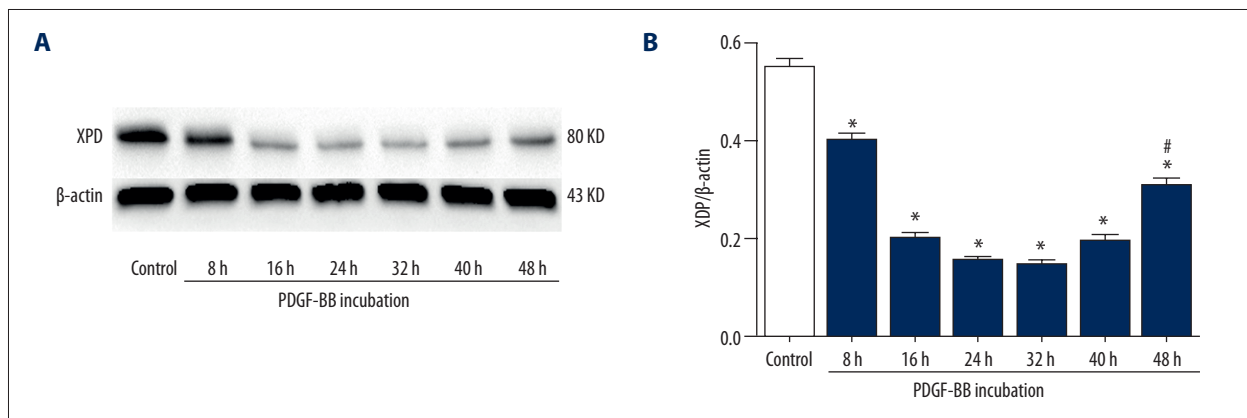


Figure 2. XPD protein expression levels in HUVMSCs in response to PDGF-BB. * $P < 0.01$ vs. control group, # $P < 0.01$ vs. PDGF treatment 24-h group.

1: 1000, GSK3 β 1: 1000, β -actin 1: 1000, Tubulin 1: 1000) at 4°C overnight. Membranes were then incubated with peroxidase-conjugated goat anti-rabbit or -mouse IgG (1: 5000) for 2 h at room temperature. The signals were visualized with the ECL detection system. Quantification of protein bands was analyzed by Quantity One software (Bio-Rad, USA).

Statistical analysis

Results are expressed as means \pm standard deviation (SD). Each experiment was replicated 3 times. Statistical differences between groups were assessed by one-way ANOVA followed by the Student-Newman-Keul post hoc test, as applicable. A value of $p < 0.05$ was considered significant.

Results

Optimization of the concentration of PDGF-BB and incubation time

MTT assay results showed that the cell viability was increased in a dose-dependent manner within the concentration of PDGF-BB at 10 ng/ml, 30 ng/ml, and 80 ng/ml (Figure 1A), and also increased in a time-dependent manner within 0 h, 8 h, 16 h, 24 h, 32 h, 40 h, and 48 h of incubation time (Figure 1B). Therefore, the concentration of PDGF-BB at 30 ng/ml via an additional 24-h incubation was chosen for the subsequent experiments.

XPD protein expression levels in HUVMSCs exposed to PDGF-BB

Western blot analysis showed that the expression of XPD protein decreased in a time-dependent manner with PDGF-BB incubation and reached the low point at 24 h and 32 h (Figure 2). Compared with the PDGF treatment 24 h group, XPD expression

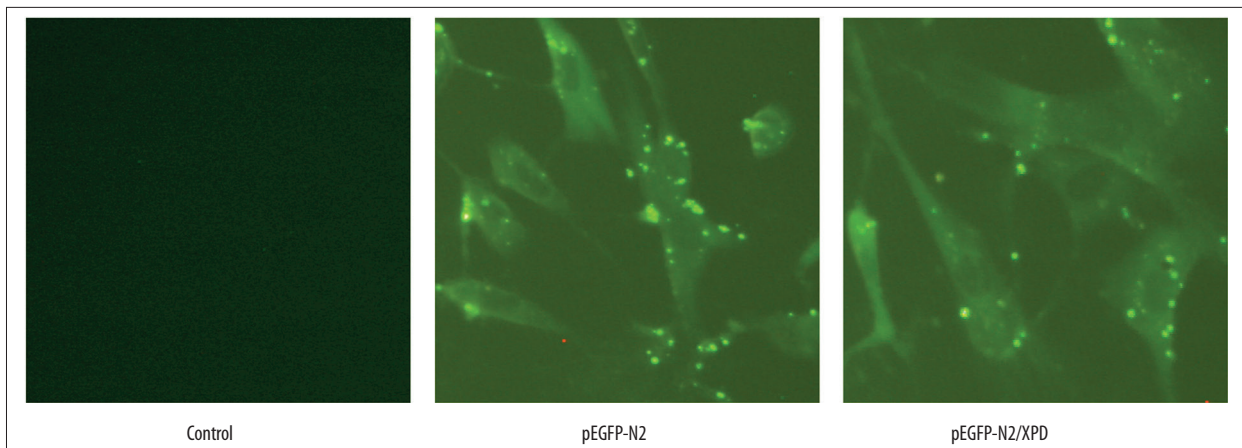


Figure 3. GFP in VSMCs was observed under a fluorescence microscope.

was higher in HUVMSCs with 48-h PDGF-BB incubation. The relationship between the expression of XPD protein and time is a “u” curve. Therefore, 24 h of PDGF-BB was selected to investigate the role of XPD in PDGF-BB-induced HUVMSC proliferation in subsequent experiments.

Overexpression of XPD inhibited HUVMSC proliferation induced by PDGF-BB

MTT assay showed a significant increase of cell viability in HUVMSCs exposed to PDGF-BB for 24 h while the expression of XPD protein reached the low point. To examine whether XPD would lead to cell cycle arrest, HUVMSCs were transfected with recombinant plasmid pEGFP-N2/XPD, followed by PDGF-BB incubation for 24 h. Then, cell cycle was detected by flow cytometry.

The expression of GFP was observed under a fluorescence microscope (Figure 3) at 24 h after transfection. XPD protein level in the pEGFP-N2/XPD group was significantly higher than in the control group ($P < 0.01$) (Figure 4A, 4B), which suggests that XPD protein level in the pEGFP-N2/XPD group was overexpressed. As shown in Table 1, flow cytometry results indicated that overexpression of XPD increased the number of cells blocked in G1 phase ($P < 0.01$), decreased number of cells blocked in S phase ($P < 0.05$), and inhibited PDGF-BB, promoting G1/S progression (Figure 4C).

The influence on CDK4 and cyclin D1 expression in VSMCs with XPD overexpression and PDGF-BB exposure

To test whether XPD expression is linked with HUVMSC proliferation, CDK4 and cyclin D1, which were associated with the G1 phase of the cell cycle, were detected by Western blot. Results showed XPD overexpression suppressed the protein expression of CDK4 (0.20 ± 0.01 , vs. 0.54 ± 0.03 , $P < 0.01$ vs. control group) and cyclin D1 (0.22 ± 0.02 vs. 0.29 ± 0.01 , $P < 0.01$

vs. control group). Pretreatment with pEGFP-N2/XPD inhibited the accelerating effect of PDGF-BB on protein expression of CDK4 (0.46 ± 0.02 vs. 0.80 ± 0.03 , $P < 0.01$ vs. PDGF-BB group) and cyclin D1 (0.23 ± 0.01 vs. 0.51 ± 0.02 , $P < 0.01$ vs. PDGF-BB group) in VSMCs (Figure 5). These results suggest XPD can regulate cell proliferation by suppressing the expression of CDK4 and cyclin D1.

GSK3 β mediates the inhibitory effects of XPD on VSMC proliferation induced by PDGF-BB

To identify the relationship between XPD and cyclin D1, we investigated protein expression levels of GSK3 β and p-GSK3b(Ser9). Results showed that XPD overexpression decreased the expression of p-GSK3 β (0.11 ± 0.02 vs. 0.33 ± 0.02 , $P < 0.01$ vs. control group), and upregulating XPD inhibited the accelerating effect of PDGF-BB on p-GSK3 β (0.17 ± 0.005 vs. 0.62 ± 0.01 , $P < 0.01$ vs. PDGF-BB group) (Figure 6A). However, there was no significant difference in GSK3 β expression among all groups (Figure 6A). These results suggest that XPD increased the activity of GSK3 β by diminishing the phosphorylation of GSK3 β . In order to define the critical role of GSK3 β in the inhibitive function of XPD on VSMC growth, VSMCs were treated with the GSK3 β inhibitor SB216763 for 24 h after transfection with pEGFP-N2/XPD. Results revealed SB216763 inhibited the reduction effect of XPD on CDK4 (0.28 ± 0.01 vs. 0.16 ± 0.01 , $P < 0.01$ vs. pEGFP-N2/XPD group) and cyclin D1 (0.22 ± 0.01 vs. 0.16 ± 0.01 , $P < 0.01$ vs. pEGFP-N2/XPD group) in VSMCs (Figure 6B).

Discussion

It is well known that VSMC abnormal proliferation plays a crucial role in intimal thickening [13,14]. A common factor in the inappropriate VSMC proliferation is phosphorylation of proteins through activation of MAPK, AKT, and ERK pathways [15–17].

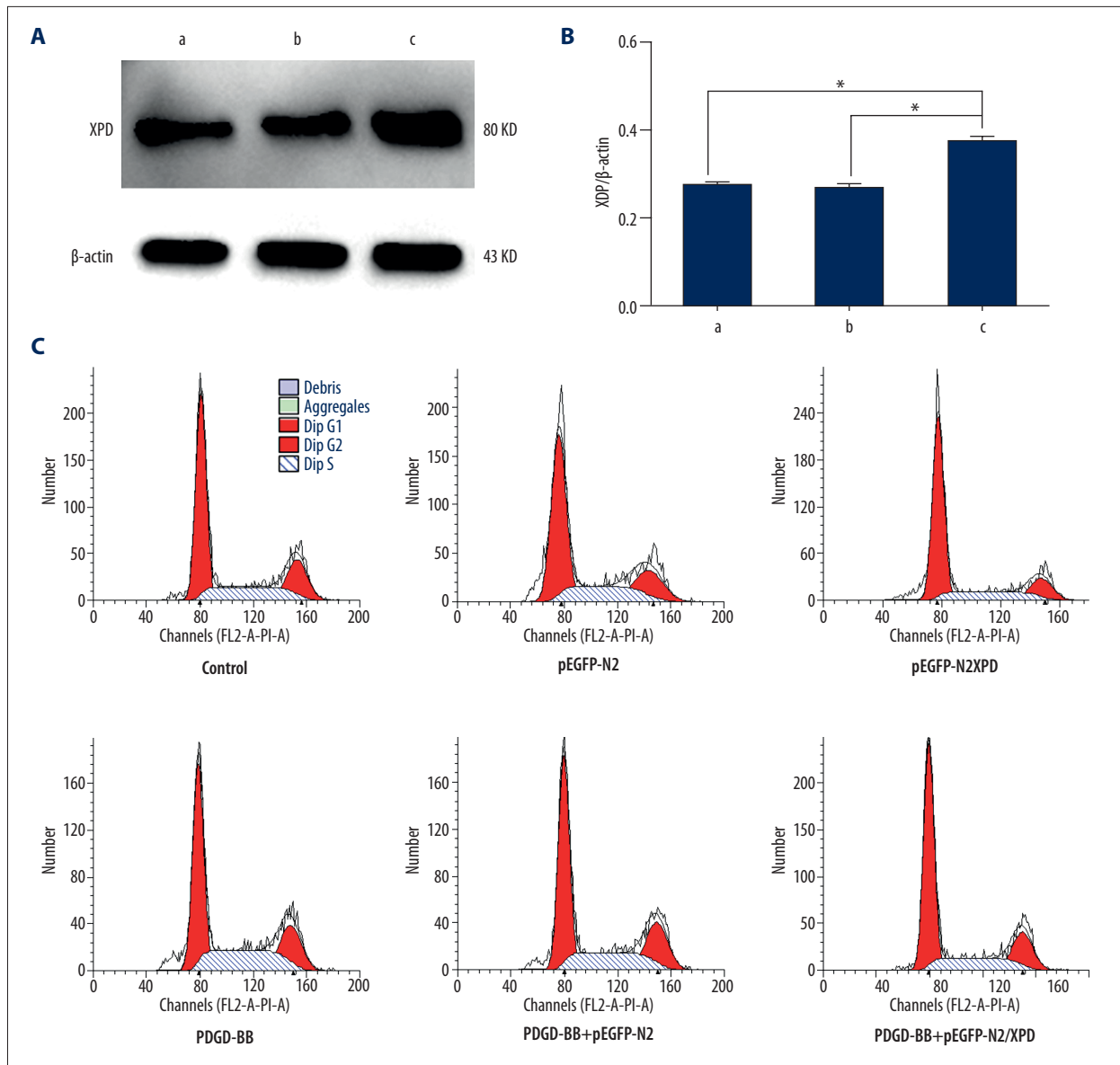


Figure 4. Overexpression of XPD inhibited HUVSMC proliferation induced by PDGF-BB. (A, B) After plasmid transfection, XPD protein expression level was detected by Western blot analysis. a. control group, b. pEGFP-N2 group, c. pEGFP-N2/XPD. * $P < 0.01$. (C) Flow cytometry analysis of each group.

Table 1. The cell cycle distribution of each group.

Groups	G ₁ (%)	S (%)	G ₂ (%)
Control group	54.28±0.77	24.78±0.68	20.94±0.25
pEGFP-N2group	54.31±0.65	24.67±0.44	21.02±0.31
pEGFP-N2/XPD group	62.31±1.26*	21.28±0.34**	16.41±1.15
PDGF-BB group	48.08±1.78*	31.55±0.38**	20.70±0.45
PDGF-BB + pEGFP-N2 group	51.05±0.59	26.93±0.43	22.03±0.63
PDGF-BB + pEGFP-N2/XPD group	58.18±0.42#	21.03±0.90#	20.78±1.30

* $P < 0.01$ vs. control group, ** $P < 0.05$ vs. control group, # $P < 0.01$ vs. PDGF-BB group.

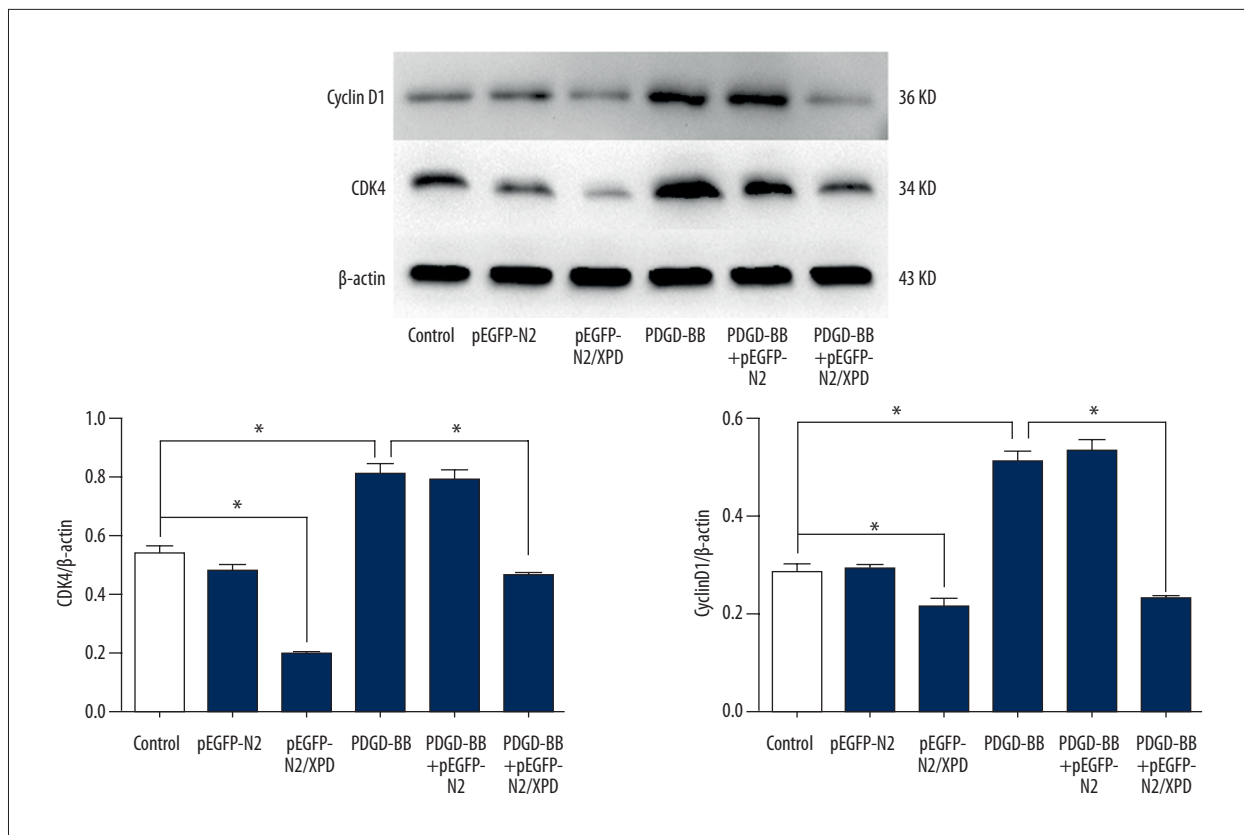


Figure 5. The influence of protein expression of CDK4 and cyclin D1 in HU VSMCs with XPD overexpression and PDGF-BB incubation.
* $P < 0.01$.

Hypersecretion of PDGF has been associated with VSMC proliferation in atherogenesis [18]. PDGF stimulation can enhance the expression of CDK4 and cyclin D1 [19–21], which has been confirmed by our study (Figure 4).

XPD, a DNA helicase with 5'-3' polarity, also participate in regulation of the cell cycle. XPD negatively regulates the cell cycle function of CDK7, while the downregulation of XPD resulted in increased cell proliferation [11]. *In vitro*, XPD contributes to accelerating cell apoptosis by increasing the expression of p53 and decreasing the expression of c-Myc and CDK2 [12]. To better understand XPD's role in VSMC proliferation, we investigated the cellular effects of XPD in VSMCs incubated with PDGF *in vitro*. We observed a time-dependent increase of cell viability in VSMCs incubated with PDGF *in vitro*, accompanied by decreasing effect of XPD. The results indicated that XPD serves as a cell proliferation inhibitor in VSMCs. However, compared with the PDGF treatment 24-h group, XPD expression was higher but cell viability has no significant difference in VSMCs exposed to PDGF for 48 h, suggesting the presence of additional mechanisms in VSMC proliferation in response to atherogenic stimuli [20] and the activation of DDR pathways [22,23]. XPD is required in the NER pathway to acquire genome integrity [24]. It has been reported that XPD is localized in mitochondria, and

cells under oxidative stress have shown an enhanced aggregation of XPD into mitochondria [25].

The cell cycle is a highly regulated cell progression that leads to controlled cell division. Cells first prepare for DNA synthesis (G1 phase), replicate their DNA (S phase), prepare for mitosis (G2 phase), and undergo mitosis (M phase). Specific proteins serve as door guards at every phase to prevent cells from early entrance into the next stage of the cell cycle [26,27]. Cyclin D1 is a regulatory subunit of CDKs that regulates the G1-to-S cell cycle transition [28]. Many studies have shown that cyclin D1 activates CDK4 and CDK6 and drives cell cycle progression [29]. The cyclin D1-CDK4 complex facilitates G1/S transition [30]. A recent study has shown that CDK7 activates CDK4 to regulate G1 progression [31]. XPD decreases the activity of CDK7, which is an important stimulator of CDKs, thus suppressing cell proliferation [32]. Li reported that XPD dynamically localizes Cdk7/CAK to and away from subcellular substrates, thereby regulating cell proliferation, proving that the function of XPD in cell cycle regulation is independent of transcription or NER [33]. Accordingly, we supposed that the exogenous XPD may inhibit inappropriate proliferation of VSMCs. Our results showed XPD overexpression significantly augmented cell cycle arrest and attenuated CDK4 and cyclin D1

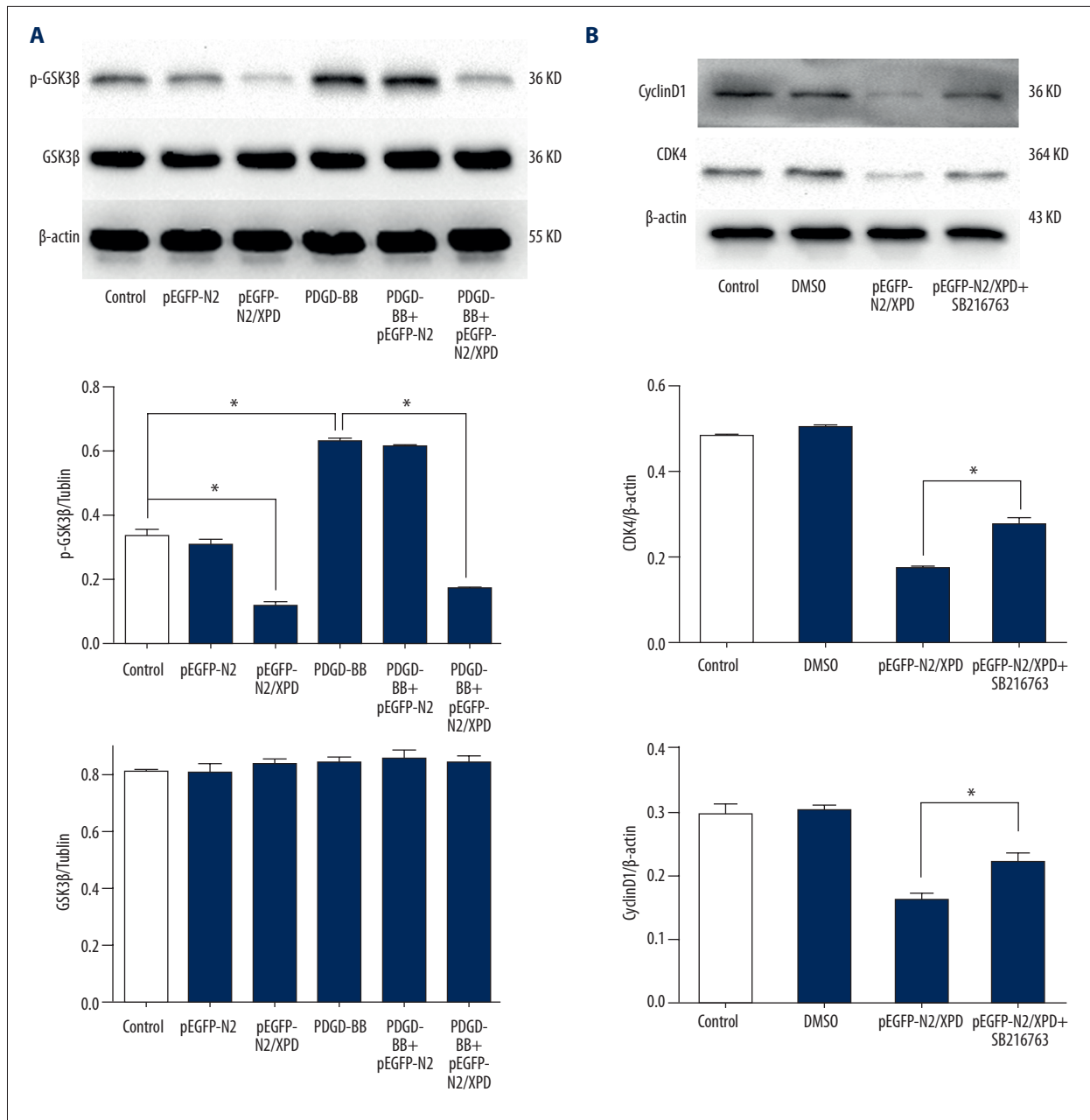


Figure 6. GSK3 β mediated the inhibitory effects of XPD on HUVMC proliferation induced by PDGF-BB. * $P < 0.01$ (A) The influence on p-GSK3 β and GSK3 β in HUVMCs with XPD overexpression and PDGF-BB exposure. (B) SB216763 inhibited the reduction effect of XPD on CDK4 and cyclin D1.

in VSMCs. Transfection of pEGFP-N2/XPD prior to PDGF incubation significantly suppressed G1/S transition and attenuated CDK4 and cyclin D1 in VSMCs with PDGF treatment. These results are consistent with previous studies reporting that CDK4 activation is prevented by CDK7 inhibition [34]. Cyclin D1/CDK4 complexes phosphorylate retinoblastoma 1(RB1), which releases E2F proteins, leading to the transactivation of genes required for the G1/S transition [35].

The signaling mechanism underlying the inhibitory effects of XPD on VSMC proliferation remain uncharacterized. GSK3 β is a multifunctional serine/threonine kinase and is associated with many cell processes, including cell proliferation, cell differentiation, cell migration, and the regulation of multiple transcription [36,37]. Phosphorylated GSK-3 β participate in mPTP opening during ischemia [36]. GSK3 β can be modulated by signal transduction pathways involved in cell processes, such as PKA, PKB, PKC, PKG, PI3K, and ERK1/2, leading to

orderly cellular functions. It is inactivated by the phosphorylation of Ser9 [38,39]. PICART1, a novel p53-inducible tumor-suppressor lncRNA, suppressed cell proliferation through the AKT/GSK3 β / β -catenin signaling cascade [40]. Wang reported that XPD contributes to accelerating cell apoptosis by increasing the expression of p53 [12]. *In vitro*, silencing of XPD led increased c-Myc expression [41]. It has been reported that c-Myc accelerates the expression of CDK4 and cyclin D1, and thus promotes G1/S transition [41,42]. GSK3 β also can trigger the degradation of c-Myc by phosphorylating the specific site Thr58 [38]. Accordingly, we hypothesized that XPD regulates VSMC proliferation through activating GSK3 β . Our results showed that XPD diminished the phosphorylation of GSK3 β , while the GSK3 β inhibitor SB216763 inhibited the reduction effect of XPD on CDK4 and cyclin D1. These results suggest the activation of GSK3 β is critical in the interaction. It would be of interest to elucidate the underlying mechanisms by which XPD induces cell cycle arrest by decreasing the phosphorylation of GSK3 β .

References:

- Isakson BE, Best AK, Duling BR: Incidence of protein on actin bridges between endothelium and smooth muscle in arterioles demonstrates heterogeneous connexin expression and phosphorylation. *Am J Physiol Heart Circ Physiol*, 2008; 294(6): H2898–904
- Gomez D, GK Owens: Smooth muscle cell phenotypic switching in atherosclerosis. *Cardiovasc Res*, 2012; 95: 156–64
- Doran AC, Meller N, McNamara CA: Role of smooth muscle cells in the initiation and early progression of atherosclerosis. *Arterioscler Thromb Vasc Biol*, 2008; 28: 812–19
- Spin JM, Maegdefessel L, Tsao PS: Vascular smooth muscle cell phenotypic plasticity: Focus on chromatin remodelling. *Cardiovasc Res*, 2012; 95: 147–55
- Slater SC, Koutsouki E, Jackson CL, Bush RC, Angelini GD, Newby AC, George SJ: R-cadherin: beta-catenin complex and its association with vascular smooth muscle cell proliferation. *Arterioscler Thromb Vasc Biol*, 2004; 24: 1204–14
- Cervelli T, Borghini A, Galli A, Andreassi MG: DNA damage and repair in atherosclerosis: Current insights and future perspectives. *Int J Mol Sci*, 2012; 13(12): 16929–44
- Durik M, Kavousi M, van der P et al: Nucleotide excision DNA repair is associated with age-related vascular dysfunction. *Circulation*, 2012; 126: 468–78
- Kraemer KH, Patronas NJ, Schiffmann R: Xeroderma pigmentosum, trichothiodystrophy and Cockayne syndrome: A complex genotype-phenotype relationship. *Neuroscience*, 2001; 145: 1388–96
- Andressoo JO, Hoesjmakers JH, de WH: Nucleotide excision repair and its connection with cancer and ageing. *Adv Exp Med Biol*, 2005; 570: 45–83
- Cameroni E, Stettler K, Suter B: On the traces of XPD: Cell cycle matters – untangling the genotype-phenotype relationship of XPD mutations. *Cell Div*, 2010; 5: 24–43
- Chen J, Larochelle S, Li X, Suter B: Xpd/Erc2 regulates CAK activity and mitotic progression. *Nature*, 2003; 424: 228–32
- Wang H, Xiong G, Zhang J et al: The role of XPD in cell apoptosis and viability and its relationship with p53 and cdk2 in hepatoma cells. *Med Oncol*, 2012; 29: 161–67
- Zhao Y, Biswas SK, McNulty PH: PDGF-induced vascular smooth muscle cell proliferation is associated with dysregulation of insulin receptor substrates. *Am J Physiol Cell Physiol*, 2011; 300: 1375–85
- Keramati AR, Rajvir S, Aiping L et al: Wild-type LRP6 inhibits, whereas atherosclerosis-linked LRP6. *Proc Natl Acad Sci USA*, 2011; 108: 1914–18
- Slater SC, Koutsouki E, Jackson CL et al: R-cadherin: beta-catenin complex and its association with vascular smooth muscle cell proliferation. *Arterioscler Thromb Vasc Biol*, 2004; 24: 1204–10
- Tsaousi A, Williams H, Lyon CA et al: Wnt4/-catenin signaling induces VSMC proliferation and is associated with intimal thickening. *Circ Res*, 2011; 108: 427–36
- Joshi CN, Martin DN, Shaver P et al: Control of vascular smooth muscle cell growth by Connexin 43. *Front Physiol*, 2012; 3: 220–33
- Kawai KK, Owens GK: Multiple repressor pathways contribute to phenotypic switching of vascular smooth muscle cells. *Am J Physiol Cell Physiol*, 2007; 292: C59–69
- Findeisen HM, Gizard F, Zhao Y et al: Epigenetic regulation of vascular smooth muscle cell proliferation and neointima formation by histone deacetylase inhibition. *Arterioscler Thromb Vasc Biol*, 2011; 31: 851–60
- Park ES, Yoo JM, Lim Y et al: Inhibitory effects of docetaxel on platelet-derived growth factor (pdgf)-bb-induced proliferation of vascular smooth muscle cells through blocking pdgf-receptor beta phosphorylation. *J Pharmacol Sci*, 2011; 116: 204–13
- Bedel A, Negre-Salvayre A, Heeneman S et al: E-cadherin/beta-catenin/t-cell factor pathway is involved in smooth muscle cell proliferation elicited by oxidized low-density lipoprotein. *Circ Res*, 2008; 103: 694–701
- Sobczuk A, Poplawski T, Blasiak J: Polymorphisms of DNA repair genes in endometrial cancer. *Pathol Oncol Res*, 2012; 18: 1015–20
- Srivastava K, Srivastava A, Kumar A, Mittal B: Gallbladder cancer predisposition: A multigenic approach to DNA-repair, apoptotic and inflammatory pathway genes. *PLoS One*, 2011; 6: 16449–57
- Chen J, Suter B: Xpd, a structural bridge and a functional link. *Cell Cycle*, 2003; 2: 503–6
- Liu J, Fang HB, Chi ZH et al: XPD localizes in mitochondria and protects the mitochondrial genome from oxidative DNA damage. *Nucleic Acids Res*, 2015; 43: 5476–88
- Sherr CJ: Cancer cell cycles. *Science*, 1996; 274: 1672–77
- Chen WC, Wu PH, Phillip JM et al: Functional interplay between the cell cycle and cell phenotypes. *Integr Biol (Camb)*, 2013; 5: 523–34
- Shimura T, Hamada N, Sasatani M et al: Nuclear accumulation of cyclin D1 following long-term fractionated exposures to low-dose ionizing radiation in normal human diploid cells. *Cell Cycle*, 2014; 13: 1248–55
- Jirawatnotai S, Hu Y, Livingston DM, Sicinski P: Proteomic identification of a direct role for cyclin d1 in DNA damage repair. *Cancer Res*, 2012; 72: 4289–93

Conclusions

XPD participates in the regulation of VSMC proliferation. XPD induces VSMC cell cycle arrest, and the activation of GSK3 β plays a crucial role in the inhibitory effect of XPD on VSMC proliferation.

Limitations

There are several limitations to this study. One is that we only studied an *in vitro* cell model. Although we showed that the inhibitory effect of XPD on VSMC proliferation may be mediated by GSK3 β , we did not examine the molecular mechanism(s) by which XPD affects the phosphorylation of GSK3 β .

Conflicts of interest

None.

30. Li Z, Shen J, Wu W et al: Leptin Induces Cyclin D1 expression and proliferation of human nucleus pulposus cells via JAK/STAT, PI3K/Akt and MEK/ERK pathways. *PLoS One*, 2012; 7: 53176–87
31. Schachter MM, Merrick KA, Larochelle S et al: A Cdk7-Cdk4 T-Loop phosphorylation cascade promotes G1 progression. *Mol Cell*, 2013; 50: 250–60
32. Vervoorts J, Luscher-Firzlaff J, Luscher B: The ins and outs of MYC regulation by posttranslational mechanisms. *J Biol Chem*, 2006; 281: 34725–29
33. Li XM, Urwyler O, Suter B: Drosophila Xpd regulates Cdk7 localization, mitotic kinase activity, spindle dynamics, and chromosome segregation. *PLoS Genet*, 2010; 6: e1000876
34. Bisteau X, Paternot S, Colleoni B et al: LCDK4 T172 phosphorylation is central in a CDK7-dependent bidirectional CDK4/CDK2 interplay mediated by p21 phosphorylation at the restriction point. *PLoS Genet*, 2013; 9: 1003546
35. Guo T, Chai X, Liu Q et al: Downregulation of P16 promotes cigarette smoke extract-induced vascular smooth muscle cell proliferation via preventing G1/S phase transition. *Exp Ther Med*, 2017; 14: 214–20
36. Karrasch T, Spaeth T, Allard B, Jobin C: PI3K-dependent GSK3 β (Ser9)-phosphorylation is implicated in the intestinal epithelial cell wound-healing response. *PLoS One*, 2011; 6: 26340–49
37. Kaidanovich-Beilin O, Woodgett JR: GSK-3: Functional insights from cell biology and animal models. *Front Mol Neurosci*, 2011; 4: 40
38. Jirawatnotai S, Hu Y, Livingston DM, Sicinski P: Proteomic identification of a direct role for Cyclin D1 in DNA damage repair. *Cancer Res*, 2012; 72: 4289–93
39. Das S, Steenbergen C: Mitochondrial adenine nucleotide transport and cardioprotection. *J Mol Cell Cardiol*, 2012; 52: 448–53
40. Cao Y, Lin M, Bu Y et al: p53-inducible long non-coding RNA PICART1 mediates cancer cell proliferation and migration. *Int J Oncol*, 2017; 50: 1671–82
41. Mateyak MK, Obaya AJ, Sedivy JM: c-Myc regulates cyclin D-Cdk4 and -Cdk6 activity but affects cell cycle progression at multiple independent points. *Mol Cell Biol*, 1999; 19: 4672–83
42. Zhang J, Song N, Zang D et al: c-Myc promotes tumor proliferation and antiapoptosis by repressing p21 in rhabdomyosarcomas. *Mol Med Rep*, 2017; 16: 4089–94