Mechanical stretch induces the apoptosis regulator PUMA in vascular smooth muscle cells

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Aims

The expression of PUMA (p53-up-regulated modulator of apoptosis), an apoptosis-regulating gene, increases during endoplasmic reticulum stress. The mechanisms by which cyclic stretch influences the regulation of PUMA in vascular smooth muscle cells (VSMCs) during apoptosis remain unclear. We hypothesized that cyclic stretch enhances PUMA expression in VSMCs undergoing apoptosis.

Methods and results

Human VSMCs grown on a Flexcell I flexible membrane base were stretched via vacuum to 20% of elongation at a frequency of 1 Hz. An in vivo model of volume overload with aorta-caval shunt and pressure overload with aortic banding in adult rats was used to study PUMA expression. Cyclic stretch markedly enhanced PUMA protein and gene expression after stretch. Addition of c-jun N-terminal kinase (JNK) inhibitor SP600125 and interferon-γ (IFN-γ) antibody 30 min before stretch inhibited PUMA expression. Gel shift assay demonstrated that stretch increased the DNA binding activity of interferon regulatory factor-1 (IRF-1). SP600125, JNK small interfering RNA, and IFN-γ antibody attenuated the DNA binding activity induced by stretch. PUMA-Mut plasmid, SP600125, and IRF-1 antibody attenuated the promoter activity. Stretch increased secretion of IFN-γ from VSMCs, and conditioned media from stretched VSMCs increased PUMA protein expression. The in vivo model of aorta-caval shunt and aortic banding also showed increased PUMA protein expression in the aorta.

Conclusion

Cyclic mechanical stretch increases PUMA expression in cultured human VSMCs. The PUMA expression induced by stretch is mediated by IFN-γ, JNK, and IRF-1 pathways. These findings suggest that PUMA is an important mediator in VSMC apoptosis induced by stretch.

Keywords

Apoptosis • Smooth muscle cell • p53-Up-regulated modulator of apoptosis • Cyclic stretch

1. Introduction

Although prevention strategies for atherosclerosis have significantly progressed, atherosclerosis is still a major and increasing health concern in developed countries. Thus, the development of novel therapeutic agents for patients with atherosclerosis remains a major research priority.1 Atherosclerosis is characterized by lipid accumulation within vessel walls, inflammation, and apoptosis of both macrophages and vascular smooth muscle cells (VSMCs).2 VSMC apoptosis plays an important role in both normal and pathological remodelling of vessel walls.3 VSMC apoptosis has been increasingly implicated in both the development and the outcome of atherosclerotic disease.4 Accelerated VSMC apoptosis may induce thinning of fibrous cap (atheroma caused by vessel inflammation, resulting in lumen narrowing) and is very important in plaque stability.5 VSMC apoptosis is rare in normal blood vessels; however, apoptosis is increased in unstable human atherosclerotic plaques. Apoptosis of VSMCs is sufficient to induce plaque vulnerability in atherosclerosis.6 Therefore, reducing VSMC apoptosis is a promising strategy for attenuating plaque instability and atherosclerosis.7

Endoplasmic reticulum (ER)-initiated apoptosis is implicated in the pathophysiology of plaque rupture and atherosclerosis in animal and human studies.8 One component of the ER stress-mediated apoptosis pathway is the p53-up-regulated modulator of apoptosis (PUMA), also known as Bcl-2 binding component 3.9 PUMA is a Bcl-2 homology 3 (BH3)-only Bcl-2 family member and is similar to other BH3-only
proteins, which serve as proximal signalling molecules that transduce death signals to the mitochondria. Previously, Yu and Zhang have demonstrated that PUMA is a critical mediator of p53-dependent and -independent apoptosis in response to various stimuli in several tissues and cells. 10 It has been implicated in the pathophysiological mechanisms of several diseases, including cancer, acquired immunodeficiency syndrome and ischaemic brain disease. 11

Mechanical force overload is capable of inducing inflammatory mediators and is known to cause ventricular hypertrophy. 12 Furthermore, using a cyclic strain system on cultured cells subjects them to repetitive stretching and relaxation at rates comparable to dynamic stretch overload in vivo. This system has been applied widely in studying the molecular mechanisms of gene expression and signal transduction in many cell types. 13 Moreover, it has been demonstrated that cyclic stretch could induce apoptosis of cardiovascular cells. 14 PUMA plays an important role in apoptosis of cardiomyocytes following ER stress and ischaemia–reperfusion. 15 Additionally, PUMA is involved in apoptosis of VSMCs. 16 However, few studies have reported on whether cyclic stretch affects the expression of PUMA in VSMCs undergoing apoptosis. Interferon regulatory factor-1 (IRF-1) also plays an important role in apoptosis. In response to interferon-γ (IFN-γ), PUMA is trans-activated by IRF-1. 17 Thus, the aim of the present study was to test the hypothesis that cyclic stretch enhances PUMA expression in VSMCs undergoing apoptosis. We also investigated the role of IRF-1 in mediating the stretch-induced expression of PUMA.

2. Methods

2.1 Culture of VSMCs

Human coronary artery VSMCs were purchased from PromoCell (Heidelberg, Germany). Cells were cultured in smooth muscle cell growth medium 2 containing 20% fetal calf serum, 0.1 mmol/L non-essential amino acids, 1 mmol/L sodium pyruvate, 4 mmol/L l-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in an atmosphere of 5% CO2–95% air in a humidified incubator. When confluent, VSMC monolayers were passaged every 6–7 days after trypsinization and were used for experimentation from the third to sixth passages. These third to sixth passage cells were then cultured in Flexcell I flexible membrane dishes coated with Collagen I (FLEX/CULTURE PLATES COLLAGEN I, Flexcell International, Hillborough, NC, USA) in smooth muscle cell growth medium 2 containing 0.5% fetal calf serum, and the cells were incubated for an additional 2 days to render them quiescent prior to initiating each experiment.

2.2 In vitro cyclic stretch of cultured VSMCs

The Flexcell FX-2000 strain unit (Flexcell International, Hillborough, NC, USA) consists of a vacuum unit linked to a valve controlled by a computer program. VSMCs cultured on the flexible membrane base were subjected to cyclic stretch produced by this computer-controlled application of sinusoidal negative pressure with a peak level of ~15 kPa at a frequency of 1 Hz for various periods of time.

2.3 Western blot analysis

A western blot was performed as previously described. 18 Antibodies used for the western blot are described in detail in the Supplementary material online.

2.4 Reverse transcription-PCR

Total RNA was isolated from VSMCs using the single-step acid guanidinium thiocyanate–phenol–chloroform extraction method. The method for reverse transcription-PCR (RT-PCR) is further described in detail in the Supplementary material online.

2.5 Real-time PCR

The primers used were as follows: PUMA, 5′-d(CCCTGGAGGGTT CCTGTACAA)-3′ (forward) and 5′-d(CTCTGGCCTGCGGTA A)-3′ (reverse); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5′-d(CATCACCATCTTCCAGGAGC) (forward) and 5′-d(GGATGATGTCGGGCTGCC)-3′ (reverse). Details of the procedures are further described in the Supplementary material online.

2.6 Electrophoretic mobility shift assay (EMSA)

Nuclear protein concentrations from cultured VSMCs were determined by Bio-Rad (Hercules, CA, USA) protein assay. Consensus and control oligonucleotides (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were labelled by polynucleotides kinase incorporation of [γ32P]ATP. After the IRF-1 was radiolabelled, the nuclear extracts (4 μg of protein in 2 μL of nuclear extract) were mixed with 20 pmol of the appropriate [γ32P]ATP-labelled consensus or mutant oligonucleotide in a total volume 20 μL for 30 min at room temperature. The samples were then resolved on a 4% polyacrylamide gel. Gels were dried and imaged by autoradiography. Controls were performed in each case with mutant or cold oligonucleotides to compete with labelled sequence.

2.7 RNA interference

VSMCs were transfected with 800 ng small interfering RNA (siRNA) of PUMA or c-jun N-terminal kinase (JNK; Dharmaco, Lafayette, CO, USA). PUMA or JNK siRNA are target-specific 19 or 21 nucleotide siRNAs according to a computer program provided by Dharmaco. The PUMA and JNK targeted base sequences were as follows: sense, 5′-AAAGAGAGGCAACAUCAAGCA and 5′-CGUGAAUUAUGGUCU GUGdGdTd-3′, respectively; and antisense, 5′-PACAGAAGAGAC AACAUGCA and 5′-CACAGACCAUUAAUCACdCd-3′, respectively. Green fluorescent protein (GFP) siRNA was used as a negative control, with the following base sequences: sense, 5′-P-GGCUCAGCU CCAGGAGGCCAC-3′ and antisense, 5′-PUGGCUCCUGGAC UAGCCUU-3′ (Dharmacon). After overnight incubation, cells were stretched and subjected to analysis by western blot, EMSA, immunohistochemistry and detection of apoptosis.

2.8 Promoter activity assay

A human PUMA promoter construct was generated as previously described (human PUMA: −2183 to −1490, containing an IRF-1 binding site 17 and −318 to −15, containing a p53 binding site 15). The plasmids were transfected into VSMCs using a low-pressure-accelerated gene gun (Bioware Technologies, Taipeh, Taiwan), essentially following the protocol from the manufacturer. Test plasmid (2 μg) and control plasmid (pGL4-Renilla luciferase; 0.02 μg) were co-transfected with a gene gun in each well, and then replaced with normal cultured medium. Following 6 h of cyclic stretch, cell extracts were prepared for the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) and measured for dual luciferase activity by luminometer (Turner Designs, Sunnyvale, CA, USA).

2.9 Measurement of IFN-γ concentrations

Conditioned medium from VSMCs subjected to cyclic stretch and medium from control (unstretched) cells were collected for IFN-γ measurement. IFN-γ levels were measured using a quantitative sandwich enzyme immunoassay technique (R&D Systems, Minneapolis, MN, USA). The lower limit of detection of IFN-γ was 8.0 pg/mL. Both the intra-observer and the interobserver coefficients of variance were <10%.
2.10 Cytotoxicity studies
Cell viability after application of cyclic stretch was monitored using a trypan blue staining procedure and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay to detect for stretch-induced cell injury. Cytotoxicity studies were performed as previously described.18

2.11 Flow cytometric analysis and caspase 3 activities for apoptotic quantification
Apoptotic cells were quantified as the percentage of cells with hypodiploid DNA (sub-G1). VSMCs were fixed with 70% ethanol and treated with RNase. Then nuclei were stained with propidium iodide (Molecular Probes, Eugene, OR, USA) and fluorescein isothiocyanate–annexin V. DNA content was measured using by a FACSCalibur flow cytometer and Cell Quest software (Becton Dickinson, Franklin Lakes, NJ, USA). Ten thousand cells were counted in all assays. Measurement of caspase 3 activity is described in further detail in the Supplementary material online, Methods.

2.12 Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling assay
DNA fragmentation was determined via terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) using the ApopTag peroxidase in situ apoptosis detection kit (Chemicon International, Temecula, CA, USA). The methodology for the TUNEL assay is further described in detail in the Supplementary material online, Methods.

2.13 The aorta-caval shunt and aortic banding rat model
Aorta-caval (AV) shunt and aortic banding was performed on rats to induce volume and pressure overload, respectively. On the day of surgery, Wistar rats weighing 280–330 g were anaesthetized with 2% isoflurane, and the vena cava and aorta were exposed via abdominal mid-line incision after confirming a fully anaesthetized state (e.g. no response to toe pinching). Both procedures are further described in the Supplementary material online, Methods. The rats were killed with an overdose of isoflurane. All study protocols were approved by our Institutional Committee of Animal Care and Use (protocol number #0990816001) and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication no. 86-23, revised 1996).

2.14 Statistical analysis
All results are expressed as means ± SEM. Statistical significance was evaluated using analysis of variance (ANOVA; GraphPad Software Inc., San Diego, CA, USA). Dunnett’s test was used to compare multiple groups with a single control group. The Turkey–Kramer comparison was used for pairwise comparisons between multiple groups following the ANOVA. A value of P < 0.05 was considered as significant.

3. Results
3.1 Cyclic stretch enhances PUMA protein and mRNA expression in VSMCs
PUMA protein levels began to increase as early as 14 h after stretch to 20% of elongation, reached a maximal increase that was 3.5-fold higher than control values by 18 h, and remained elevated for up to 24 h. When VSMCs were stretched to 10% of elongation, PUMA protein levels were similar to those of control cells without stretch (Figure 1A and B). Real-time PCR demonstrated that PUMA mRNA increased significantly after 18 h of stretch at 20% of elongation (Figure 1C). These results indicate that cyclic stretch induces PUMA expression in VSMCs.

3.2 Stretch-induced PUMA protein expression in VSMCs is mediated by JNK
VSMCs were stretched by 20% for 18 h in the presence and absence of various inhibitors or siRNA to determine the signalling pathway mediating the stretch-induced increases in PUMA expression in VSMCs. As shown in Figure 2, stretch-induced increases of PUMA proteins were significantly attenuated after the addition of SP600125 (a potent, cell-permeable, selective, and reversible inhibitor of JNK) 30 min before stretching of VSMCs. PUMA proteins induced by stretch were not affected by the addition of PD98059 (a specific and potent inhibitor of extracellular signal-regulated kinase) and SB203580 (a highly specific, cell-permeable inhibitor of p38 kinase), but were partly blocked by the addition of N-acetylcycteine (a free radical scavenger). JNK siRNA was transfected into VSMCs prior to
3.3 Cyclic stretch increases IRF-1 binding activity

Cyclic stretch of VSMCs for 2–6 h significantly increased the DNA–protein binding activity of IRF-1 (Figure 3A; see Supplementary material online, Figure S2A). An excess of unlabelled IRF-1 oligonucleotide competed with the probe for binding IRF-1 protein, whereas an oligonucleotide containing a 2 bp substitution in the IRF-1 binding site did not compete for binding. Addition of SP600125, JNK siRNA, and IFN-γ antibody (5 mg/mL; purchased from R&D Systems) 30 min prior to stretch significantly inhibited the DNA–protein binding activity induced by cyclic stretch. Exogenous administration of IFN-γ to VSMCs without stretch increased IRF-1–DNA binding activity (Figure 3A; see Supplementary material online, Figure S2A). These results demonstrate that stretch enhances IRF-1 binding activity in VSMCs. Addition of JNK siRNA and SP600125 significantly decreased IFN-γ-induced IRF-1 binding activity without stretch (Figure 3B; see Supplementary material online, Figure S2B). Addition of PD98059, SB203580, angiotensin II, or tumour necrosis factor-α antibody (5 mg/mL; purchased from R&D Systems) 30 min prior to stretch had no effect on the IRF-1 binding activity induced by cyclic stretch (Figure 3B; see Supplementary material online, Figure S2B).

3.4 Cyclic stretch increases PUMA promoter activity through IRF-1

The promoter region of human PUMA was cloned, and a luciferase reporter plasmid (pGL3-Luc) was constructed to determine whether the stretch-induced expression of PUMA is regulated at the transcriptional level. The PUMA promoter construct contains cAMP response element B (CREB), nuclear factor of activated T cells (NFAT), nuclear factor-κB (NF-κB), and IRF-1 binding sites (Figure 3C). As shown in Figure 3D, a transient transfection of this reporter gene into VSMCs revealed that stretch for 6 h significantly induces PUMA promoter activation. This finding indicates that PUMA expression is induced at the transcriptional level during cyclic stretching of VSMCs. Furthermore, when the IRF-1 binding sites were mutated, the increases in promoter activity induced by stretch were abolished. Moreover, addition of the IFN-γ antibody and SP600125 resulted in an inhibition of transcription. Addition of pifithrin (p53 inhibitor) prior to stretch did not inhibit the expression of PUMA induced by cyclic stretch from VSMCs. This result indicates that increases in the expression of PUMA in VSMCs are mediated by IRF-1, and are independent of p53.

3.5 Cyclic stretch stimulates the secretion of IFN-γ from VSMCs

As shown in Figure 4A, cyclic stretch significantly increased the IFN-γ secretion from VSMCs at 1 h, and it remained elevated for 6 h. This result indicates that stretch stimulates the secretion of IFN-γ from VSMCs.

3.6 Exogenous IFN-γ increases PUMA protein expression

To investigate the direct effect of IFN-γ on PUMA expression in VSMCs, different concentrations of IFN-γ were administrated into the culture medium for 18 h. As shown in Figure 4B and C, the effects of IFN-γ on PUMA protein expression were dose dependent. Addition of the IFN-γ monoclonal antibody 30 min prior to stretch significantly blocked the expression of PUMA induced by cyclic stretch. Furthermore, addition of SP600125 and JNK siRNA attenuated IFN-γ-induced expression of PUMA. These findings suggest that IFN-γ enhances PUMA expression by cyclic stretch and that JNK is required for IFN-γ-dependent PUMA expression.
3.7 Cyclic stretch-induced apoptosis is mediated by PUMA in VSMCs

As shown in Supplementary material online, Figure S3A and B, cyclic stretch not only increased the death rate but also decreased the viability of VSMCs as determined via a cell counter and the MTT assay. These results suggest that cyclic stretch induces VSMC cell death. As shown in Figure 5A and Supplementary material online, Figure S3C, apoptosis was assessed by propidium iodide–annexin V double staining and FACS analysis. The percentage of cells stained with annexin V was elevated following stretch for 18 h and the addition of IFN-γ. Addition of PUMA and JNK siRNA before stretch significantly decreased the percentage of annexin V-positive cells induced by stretch. Caspase 3 activity was induced by cyclic stretch in VSMCs (see Supplementary material online, Figure S3D). Addition of IFN-γ (200 pg/mL) to VSMCs alone without stretch had a similar effect on caspase 3 activity to that of cyclic stretch. Conversely, addition of PUMA and JNK siRNA abolished the induction of caspase 3 activity induced by stretch. A significant increase in TUNEL-positive nuclei was present after stretch for 18 h and the addition of IFN-γ (Figure 5B; see Supplementary material online, Figure S3E). These increases in TUNEL-positive nuclei of VSMCs induced by stretch were significantly reversed by PUMA and JNK siRNA. PUMA siRNA significantly reduced the expression of PUMA protein (see Supplementary material online, Figure S4). These findings demonstrate that PUMA mediates stretch-induced apoptosis of VSMCs.

3.8 In vivo AV shunt and aortic banding increases the expression of aortic PUMA protein

AV shunt and aortic banding was performed to explore whether PUMA expression is increased during volume and pressure overload, respectively, in vivo. As shown in Figure 6, the expression of PUMA protein in rat aorta increased significantly by 5 days after induction of the AV shunt and 3 weeks after aortic banding. PUMA levels
were 3.8- and 3.6-fold greater, respectively, compared with the sham. Additionally, the AV shunt and aortic banding induced the apoptosis of VSMCs (see Supplementary material online, Figure S5). No neointima formation was found. PUMA expression was found in apoptotic cells. The increase in PUMA expression within the vessel wall after AV shunt is localized in the media area (see Supplementary material online, Figure S6). PUMA expression was found in the α-actin-positive cells, but not in the CD31-positive cells. PUMA expression was found in the media but not in the intima area.

4. Discussion

In the present study, we demonstrated the following findings: (i) cyclic stretch up-regulates PUMA expression in human VSMCs; (ii) cyclic stretch induces IFN-γ expression in VSMCs; (iii) IFN-γ acts as an autocrine factor to mediate the increase in PUMA expression induced by cyclic stretch; (iv) JNK MAP kinase and the IRF-1 transcription factor are involved in the signalling pathway of PUMA induction; (v) cyclic stretch induces apoptosis of VSMCs via PUMA; and (vi) in vivo acute haemodynamic overload increases the expression of aortic PUMA. Furthermore, PUMA was up-regulated in both a time- and load-dependent manner by cyclic stretch. Cyclic stretch of VSMCs increased the expression of PUMA at the protein and mRNA levels.

Our findings demonstrate that stretch induces IFN-γ, which, in turn, mediates cyclic stretch-induced expression of PUMA in VSMCs. Moreover, exogenous addition of IFN-γ to non-stretched VSMCs was sufficient to induce PUMA protein expression and VSMC apoptosis, similar to that observed in stretched VSMCs. These results indicate that IFN-γ acts as an autocrine mediator in response to cyclic stretch in VSMCs. Our results also demonstrated that JNK is required for IFN-γ-dependent PUMA expression. Mechanical stretch may have a direct effect on IFN-γ release. The effect of stretch on PUMA expression could be entirely attributed to IFN-γ. Rosner et al. demonstrated that IFN-γ primes VSMCs for Fas-induced apoptosis, and this may be mediated by phosphatidyl inositol 3-kinase (PI3K), Akt, and Janus-Associated Kinase-2 signal transducer and activator of transcription 1 (Jak-2/Stat1).20 Furthermore, IFN-γ-induced VSMC apoptosis was found to be accomplished by the overexpression of regulated upon activation normal T-cell expressed, and secreted (RANTES).21 These observations are consistent with our data regarding IFN-γ-induced VSMC apoptosis.

We also demonstrated that cyclic stretch up-regulates PUMA protein expression and that IRF-1–DNA binding activity required the phosphorylation of JNK, because IRF-1 binding activity was abolished by both the JNK inhibitor and JNK siRNA. SP600125 inhibited stretch-induced PUMA expression. N-Acetylcysteine, a free radical scavenger, had a partial inhibitory effect, whereas SB203580 did not have any inhibitory effect. It has been previously demonstrated that the induction of PUMA via palmitate is JNK1 dependent in primary murine hepatocytes.22 However, p38 MAP kinase also appears to be an important intracellular signalling pathway that regulates PUMA. Gomez-Lazaro et al. noted that treatment with a p38 MAPK inhibitor (SKF86002) potently inhibited PUMA expression induced by 6-Hydroxydopamine (6-OHDA).23 Furthermore, Zou et al. reported that insulin-like growth factor-1 effectively protects PC-12 neuronal cells against PUMA-mediated apoptosis enhanced by ER stress through the p38 MAP kinase pathway.24 Stretch increased JNK phosphorylation, whereas SP600125 and JNK siRNA significantly inhibited the phospho-JNK protein induced by stretch. Our data indicate that the JNK MAP kinase pathway, and not the p38 MAP kinase pathway, is the major pathway involved in the induction of PUMA by stretch and mediates the increase in binding activity of IRF-1.

In the present study, we also demonstrated that cyclic stretch-induced PUMA expression acts via the IRF-1 pathway. IRF-1 can be effectively induced in most cell types after exposure to cytokine-like TNF-α, interleukin-1 and IFN-γ. IRF-1 binds to specific sequences in the promoters of genes and controls the transcription of these genes involved in mediating the immunomodulatory, antiviral and antiinflammatory effects. Growing evidence suggests that IRF-1 plays an important role in apoptosis. Gao et al. have demonstrated that in response to IFN-γ, PUMA is transactivated by IRF-1.17 The involvement of PUMA in stress-induced apoptosis was mostly regulated by the tumour suppressor, p53.10 Wang et al. noted that the binding of nuclear p53 to the specific sites within the PUMA promoter is essential for its ability to induce DNA damage and apoptosis.25 It has been previously demonstrated that PUMA is up-regulated in response to reactive oxygen species or anoxia, and that p53 is responsible for this
Figure 5 Effect of PUMA on stretch-induced apoptosis in VSMCs. (A) VSMCs were subjected to cyclic stretch for 18 h, with the addition of 200 pg/mL IFN-γ or siRNA before stretch. Quantification of the apoptotic fractions was performed using FACScan. Cells that stain negative for both annexin V and propidium iodide are alive. Cells that stain positive for annexin V and negative for propidium iodide are undergoing apoptosis. Cells that stain positive for both annexin V and propidium iodide are in the end stage of apoptosis, called second apoptosis (n = 3). *p < 0.01 vs. control. **p < 0.01 vs. stretch 18 h. (B) Representative microscopy images of VSMCs after cyclic stretch for 18 h, addition of 200 pg/mL IFN-γ, or siRNA before being stretched then stained with a TUNEL kit. Similar results were observed in another two independent experiments. Arrows indicate TUNEL-positive cells.
It has been argued that calpain counteracts the mechanically induced excessive VSMC apoptosis through its p53 degrading properties. We previously demonstrated that cyclic mechanical stretch-induced VSMC apoptosis is mediated by GADD153 (hallmarks of unfolded protein response, which is up-regulated by the ER stress). PUMA siRNA did not inhibit stretch-mediated GADD153 expression (see Supplementary material online, Figure 3SB), indicating that the expressions of PUMA and GADD153 during stretch are not related. Morrow et al. noted that cyclic strain enhances smooth muscle cell apoptosis, at least in part, via the regulation of the Notch receptor. Moreover, Su et al. demonstrated that stretch-induced VSMC apoptosis is regulated via Bcl-2-associated death factor. In the present study, our findings suggest that the ER stress-related pro-apoptotic protein, PUMA, may be involved in VSMC apoptosis induced by mechanical stretch. Several studies have previously indicated that PUMA appears to function as a direct activator of BAX and BAK (members of Bcl-2 family), which initiate mitochondrial apoptosis and directly antagonize the well-known anti-apoptotic Bcl-2 family members. However, the correlation between PUMA and apoptosis is still poorly understood. We found that cyclic stretch enhances caspase 3 activity and that PUMA siRNA abolishes the induction of caspase 3 by stretch. This finding indicates that caspase 3 is involved in the PUMA-induced apoptosis of VSMCs during cyclic stretch.

The increase in PUMA protein expression levels after acute haemodynamic overload may contribute to the regulation of vascular repair, remodelling and atherosclerosis, all of which involve VSMC proliferation and apoptosis. Toth et al. noted that myocardial PUMA levels are increased in the ischaemia–reperfusion model and that cardiac function is significantly better in Puma−/− mice than in their wild-type littermates; however, the role of PUMA in this animal model is not well understood, and therefore, future research is warranted to resolve this issue. Although VSMCs play a crucial role in atherosclerosis, it is not known whether prevention of VSMC apoptosis alone can reduce atherosclerosis. Further studies are needed to clarify whether therapeutic targeting of PUMA will also prevent apoptosis of inflammatory cells in the vessel wall with potential detrimental outcome.

In summary, the present study demonstrates, for the first time, that cyclic stretch enhances the expression of PUMA in cultured human VSMCs. The stretch-induced increase in PUMA expression is mediated by IFN-γ, JNK MAP kinase, and IRF-1 pathways. Additional research focusing on these aspects would be of great interest and value in understanding the role of VSMC apoptosis and its influence in atherosclerosis.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

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References

Regulation of PUMA by mechanical stretch


