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RESEARCH

PKG1 promotes the HIV-induced proliferation, migration, and fibrosis of vascular smooth muscle cells of hemorrhoids

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Abstract

Background Hemorrhoids are very common in patients with human immunodeficiency virus (HIV) infection. The risk of postoperative infection is significantly greater in HIV-positive patients than in HIV-negative individuals, and the wound healing time is significantly prolonged. This study aimed to investigate the role of HIV-associated hemorrhoids from the perspective of vascular smooth muscle cell (VSMC) function.

Methods A total of 24 hemorrhoid tissue samples (note: grade IV hemorrhoids were absence) were collected and subjected to Masson staining to evaluate fibrosis in this study. mRNA and protein levels were monitored by qPCR and WB analysis, respectively. Immunofluorescence was conducted to evaluate PKG1 and α-SMA expression. To establish a cell model *in vitro*, VSMCs were stimulated with envelope glycoprotein (gp) 120, which is a type of HIV envelope protein. Cell proliferation was assessed via a CCK-8 assay and EdU staining. Moreover, a wound healing assay was performed to assess cell migration.

Results Our data confirmed that fibrosis was present in hemorrhoid tissues from HIV-infected patients and that PKG1 expression was upregulated. Moreover, the administration of HIV gp120 promoted the proliferation and migration of VSMCs. Similarly, fibrosis-related markers (α-SMA, MMP2, MMP3, and TIMP1) were markedly upregulated. However, silencing PKG1 inhibited the proliferation, migration, and expression of fibrosis-related markers in gp120-challenged VSMCs.

Conclusion The present research revealed that PKG1 regulated the proliferation, migration, and fibrosis of VSMCs, thereby exerting detrimental effects on HIV-associated hemorrhoids.

Keywords Hemorrhoids · Human immunodeficiency virus · CGMP-dependent protein kinase 1 · Fibrosis · Vascular smooth muscle cells

Introduction

Men who have sex with men (MSM) are more susceptible to human immunodeficiency virus (HIV) infection due to their unique subculture and specific sexual practices (including anal intercourse and oral sex) [1, 2]. According to data from numerous epidemiological surveys, the numbers of HIV-infected individuals are increasing, and the age of infected individuals is decreasing [3, 4]. Anorectal diseases, such as proctitis, anal fissure, anorectal abscess, anorectal fistula, and hemorrhoids, are particularly common in this unique patient population [5, 6].

Hemorrhoids, a common and frequently occurring proctological disease, are generally accompanied by vascular hyperplasia, bleeding, and edema [7, 8]. Importantly, hemorrhoids are particularly prevalent in patients with HIV infection [9]. The risk of postoperative infection is significantly greater in HIV-positive patients than in HIV-negative individuals, and

Highlights
1. Fibrosis was observed in hemorrhoid tissues from HIV-positive patients with hemorrhoids.
2. Administration of gp120 promoted fibrosis of VSMCs.
3. PKG1 expression was increased in HIV-infected patients with hemorrhoids and in gp120-challenged VSMCs.
4. Silencing PKG1 mitigated fibrosis of gp120-challenged VSMCs.

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the wound healing time is significantly prolonged [10]. This presents a great challenge for clinicians in selecting appropriate treatment strategies for HIV-positive patients with hemorrhoids. However, the development of hemorrhoids in patients living with HIV and the underlying pathophysiological mechanisms are not completely understood.

During HIV infection and viral replication, HIV-related protein products, such as envelope glycoprotein (gp) 120, are typically generated and released into the surrounding environment [11, 12]. HIV gp120 is secreted into the cell culture medium during HIV infection *in vitro*, and it is shed into the serum and tissues of HIV-infected individuals *in vivo* [13]. Increasing evidence has demonstrated that gp120 is cytotoxic to neurons, lung microvascular endothelial cells, human arterial smooth muscle cells, and umbilical vein endothelial cells, and it exerts these effects through the activation of pro-oxidative, proinflammatory, and/or proapoptotic signaling pathways [14, 15].

Previous studies on hemorrhoids have primarily focused on the role of vascular endothelial cells in angiogenesis, inflammation, and apoptosis [16–18]. Hemorrhoids are characterized by vascular lesions and hyperplasia in the anal region. In addition to vascular endothelial cells, vascular smooth muscle cells (VSMCs), a primary component of vessel walls, may also play a vital role in the progression of hemorrhoids. However, little is known about the role of VSMC dysfunction in hemorrhoids. Previous research has revealed that human VSMCs can be infected with HIV both *in vivo* and *in vitro*, thereby exacerbating coronary atherosclerosis and vasculopathy in individuals infected with HIV [19]. Therefore, we were curious about the role of VSMC function in HIV-positive patients with hemorrhoids.

cGMP-dependent protein kinase 1 (PKG1), one of the effector proteins of guanosine 3′,5′-cyclic monophosphate (cGMP), is a serine/threonine-specific protein kinase with an N-terminal regulatory domain and a C-terminal catalytic domain [20]. After transcription from the same gene, two PKG1 isoforms, PKG1α and PKG1β, are produced by alternative splicing [21]. Previous studies have confirmed that PKG1 plays an important role in healthy VSMC function, including the modulation of vasorelaxation and the contractile phenotype [22, 23]. A recent study reported that the treatment of human mucosal epithelial cells with pseudotyped HIV type-1 (HIV-1) viral particles or recombinant gp120 protein (a type of HIV envelope protein) resulted in increased PKG1 expression [24]. However, it is still unclear whether HIV can regulate PKG1 expression in gp120-treated VSMCs. If so, how does PKG1 affect the function of VSMCs?

Thus, the present research hypothesizes that the upregulation of PKG1 expression facilitates the proliferation, migration, and fibrosis of gp120-challenged VSMCs. The current research aims to investigate the role of HIV-associated hemorrhoids from the perspective of VSMC function.

Methods

Sample collection

In this study, a total of 24 male patients with hemorrhoids who underwent anorectal surgery therapy were recruited between 2021 and 2022 from the First Hospital of Changsha (Hunan, China). For the diagnosis of hemorrhoids, the guidelines for the diagnosis and treatment of hemorrhoids in China were followed. A total of 12 HIV-negative participants were included in the control group. A total of 12 HIV-positive patients were included in the hemorrhoids + HIV group. Hemorrhoid tissue samples were collected from these patients during surgical removal. This study was approved by the Ethics Committee of The First Hospital of Changsha, with signed informed consent obtained from the patients or their families. [1] Inclusion criteria include symptomatic grade II to IV hemorrhoids diagnosed during preoperative evaluation (note: none of the 24 volunteers recruited in present study suffered from Grade IV hemorrhoids); aged between 20 and 55 years; and agreed to join the study and signed the informed consent form. [2] Exclusion criteria include history of hemorrhoid surgery or perianal surgery, with a surgical anastomosis less than 3 cm from the dentate line; combination of hepatitis B virus or hepatitis C virus infections and medical history of neoplastic, hematopoietic, or autoimmune diseases; and other anal diseases (for example, anal fissure, anal cancer, anal fistula).

Masson staining

To study fibrosis in the hemorrhoid tissue samples, Masson staining was conducted. The tissue samples were fixed with 4% paraformaldehyde overnight. Next, the tissue samples were decalcified in 20% ethylenediaminetetraacetic acid, dehydrated in gradient concentrations of ethanol, and embedded in paraffin. The sections were subsequently stained with Masson's trichrome (Solarbio) following the manufacturer's instructions. Finally, images were captured under an optical microscope (Olympus, Tokyo, Japan).

Total RNA extraction and qPCR

Total RNA was extracted from hemorrhoid tissues and VSMCs via TRIzol reagent (Beyotime) following the manufacturer's instructions. cDNA synthesis was performed with a Script Reverse Transcription Reagent Kit (TaKaRa, China). A SYBR Premix Ex Taq II Kit (TaKaRa) was used for the qPCR process. The relative expression of the target genes was calculated with the $2^{-\Delta\Delta Ct}$ method. β-actin served as a reference gene.

Western blot (WB) assay

RIPA buffer (Beyotime Biotech) was used to extract total protein from the lysates of hemorrhoid tissues or VSMCs. Additionally, the cytoplasmic and mitochondrial proteins of VSMCs were isolated following the manufacturer's instructions for a commercially available cell mitochondrial isolation kit (Beyotime Biotech). A bicinchoninic acid protein assay kit (Beyotime) was used to determine protein concentrations. After that, the protein samples were separated and transferred onto PVDF membranes (Millipore, USA). Thereafter, the PVDF membranes were blocked with 5% skim milk for 1 h before they were incubated with primary antibodies against the following proteins at 4 °C overnight (Abcam, Cambridge, MA, USA): PKG1 (ab154613, 1:1000), α-SMA (ab5831, 1:1000), MMP2 (ab86607, 1:5000), MMP3 (ab53015, 1:500), TIMP1 (ab216432, 1:200), and β-actin (ab8226, 1:1000). Next, the membranes were washed with 1% TBST and then incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (1:2500) for 2 h. Relative protein expression was analyzed via ImageJ software (National Institutes of Health, USA) and normalized to the internal control β-actin.

Immunofluorescence staining

Slices of hemorrhoid tissues and VSMCs were incubated with blocking solution and then incubated with primary antibodies against PKG1 (ab154613, 1:1000, Abcam) and α-SMA (ab5831, 1:1000, Abcam). After being washed three times, the slices were incubated with an Alexa Fluor 488-conjugated anti-rabbit antibody (ab150077, 1:500, Abcam). DAPI solution was used to stain the cell nuclei. All the slices were mounted with mounting medium and observed under a microscope to count the number of PKG1- and/or α-SMA-positive cells.

Cell culture and transfection

VSMCs were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were maintained in DMEM (Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific) and 1% antibiotics (Beyotime, Shanghai, China) at 37 °C with 5% CO_2. To knockdown PKG1 in VSMCs, adenoviruses carrying short hairpin RNA targeting PKG1 (sh-PKG1-1 and sh-PKG1-2), and the corresponding negative controls (sh-NC) were synthesized by GenePharma (Shanghai, China). VSMCs were transfected with Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. To establish a model of HIV infection in vitro, VSMCs were stimulated with gp120 at a concentration of 100 ng/mL for 24 h according to previous studies [25, 26] with slight modifications.

Cell counting kit-8 (CCK-8) assay

The viability of the VSMCs was determined via the CCK-8 assay (US Everbright, Suzhou, China; C6005) following the manufacturer's instructions. In brief, VSMCs were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated for 24 h until they adhered to the well. Next, 10 µl of CCK-8 was added to each well, followed by 1 h of incubation at 37 °C. Finally, the absorbance was measured at a wavelength of 490 nm via a Bio-Rad 550 microplate reader (Bio-Rad).

EdU staining

Following the instructions of the EdU Apollo 567 In Vitro Imaging Kit (RibiBio, Guangzhou, China), cell proliferation was assessed. In brief, VSMCs were seeded in 24-well plates at a density of 5×10^4 cells/well and incubated for 48 h. The cells were then treated with 50 µM EdU solution for 6 h, fixed with paraformaldehyde, and permeabilized with Triton X-100. After being stained with Apollo 567, the cells were stained with DAPI solution. After that, the EdU-positive cells were counted by fluorescence microscopy.

Wound healing assay

To study the migration of VSMCs, wound healing assay was utilized. VSMCs (2×10^6 cells/well) from each group were seeded in 6-well plates, with three wells for each group, and cultured in an incubator at 37 °C and 5% CO_2 for 24 h until the cells in monolayer were plated. A sterile 200-µL tip was used to scratch the monolayer of cells, and the plate was washed with PBS, followed by culture for 24 h at 37 °C in an incubator with 5% CO_2. The cells were observed under a microscope (Olympus, Tokyo, Japan) and photographed to record wound healing after 0 h and 24 h of culture. Finally, cell mobility was calculated on the basis of changes in the wound size after 0 h and 24 h of culture.

Statistical analysis

Each experiment was performed at least three times. The data are presented as the means ± standard deviations (SDs), and statistical analyses were conducted via GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, CA, USA). Differences between two groups were analyzed via unpaired Student's *t* test. Differences among three or more groups were evaluated by one-way analysis of variance (ANOVA) followed by a Newman−Keuls post hoc test. The Benjamini−Hochberg procedure was used to adjust the *P* values. A threshold of $P < 0.05$ was defined as statistically significant.

Results

Fibrosis and upregulation of PKG1 in HIV-infected patients with hemorrhoids

First, Masson staining was conducted to observe smooth muscle cells in the vessel walls of hemorrhoid tissue samples from hemorrhoid patients with or without HIV infection. As shown in Fig. 1A, normal red smooth muscle cells (indicated by the black arrow) were observed in the vessel wall of control patients, whereas smooth muscle cells in vessel walls were absent in hemorrhoid tissue samples from HIV-infected patients. In addition, there was a significant increase in blue collagen fibers (indicated by the red arrow), indicating that HIV stimulated fibrosis in the smooth muscle cells of hemorrhoid tissues. Subsequently, PKG1 expression was measured in hemorrhoid tissue samples. As expected, PKG1 expression was significantly increased in HIV-positive patients with hemorrhoids (Fig. 1B–D). Similarly, the fluorescence intensities of both PKG1 and α-SMA were dramatically increased in hemorrhoid tissue samples from HIV-positive hemorrhoid patients (Fig. 1E–F). These data confirmed that fibrosis and upregulated PKG1 expression occurred in tissues from HIV-infected patients with hemorrhoids.

Proliferation, migration, and fibrosis were stimulated in gp120-exposed VSMCs

To further investigate whether HIV stimulates fibrosis in vitro, the VSMCs were exposed to 100 ng/mL gp120 for 24 h to establish a cell model. The results from the CCK-8 assay and EdU staining demonstrated that cell viability was dramatically increased in gp120-exposed VSMCs (Fig. 2A–C). Moreover, a wound healing assay revealed that gp120 enhanced the migration ability of VSMCs (Fig. 2D–E). Fibrosis of the VSMCs was subsequently observed after the administration of gp120. The mRNA and protein levels of fibrosis-associated markers, including α-SMA, MMP2, MMP3, and TIMP1, were significantly increased in gp120-stimulated VSMCs (Fig. 2F–N). As expected, both the mRNA and protein levels

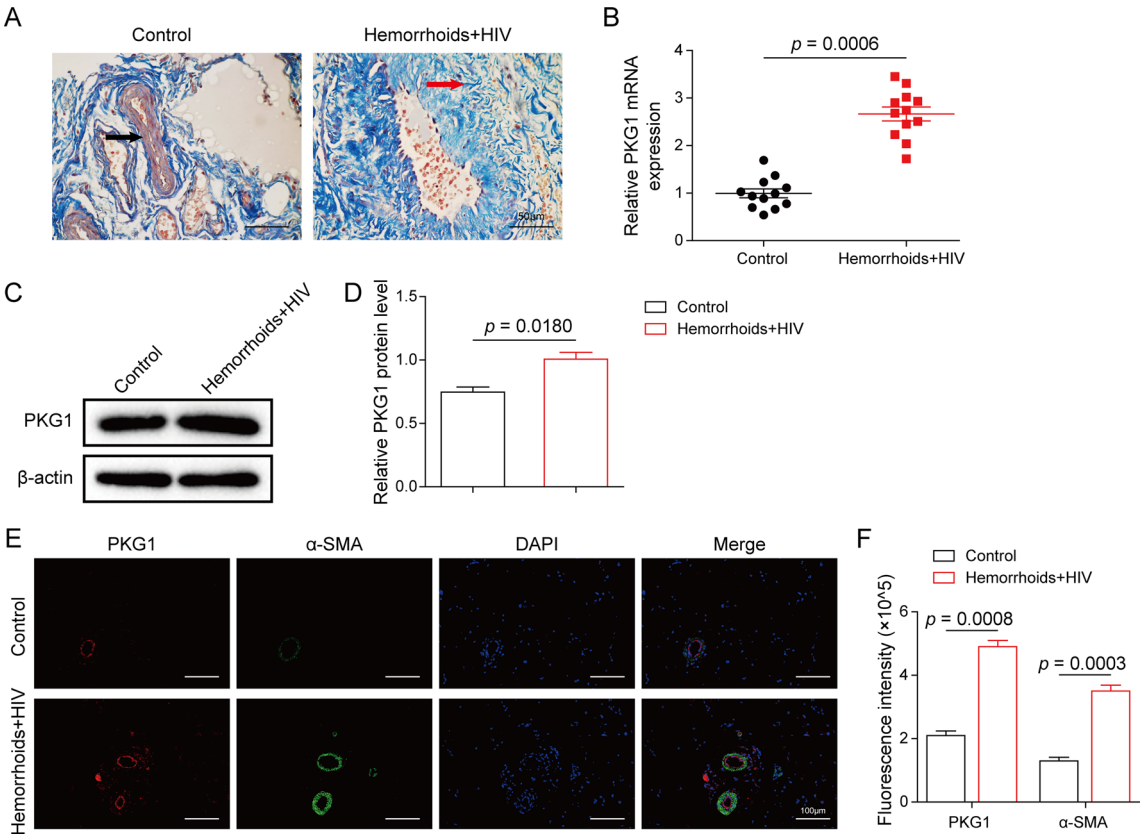

Fig. 1 Fibrosis and upregulation of PKG1 in HIV-infected patients with hemorrhoids. **A** A total of 12 HIV-negative hemorrhoid patients were included in the control group. A total of 12 HIV-positive hemorrhoids patients were included in the Hemorrhoids + HIV group. The representative images of Masson's trichrome-stained hemorrhoid tissue samples is shown (the black arrow indicates normal smooth muscle cells, while the red arrow indicates collagen fibers). **B** Results from qPCR revealed that mRNA expression of PKG1 was significantly increased in HIV-positive hemorrhoids. **C–D** The protein level of PKG1 in hemorrhoid tissue samples was measured by WB analysis. The results revealed that PKG1 was elevated in the Hemorrhoids + HIV group. **E–F** The fluorescence intensity of PKG1 and α-SMA were dramatically increased in hemorrhoid tissue samples from HIV-positive hemorrhoid patients

of PKG1 were markedly increased in gp120-treated VSMCs (Fig. 2O–Q). Immunofluorescence staining for PKG1 and α-SMA was increased in gp120-induced VSMCs (Fig. 2R). Thus, the administration of gp120 not only promoted the proliferation, migration, and fibrosis of VSMCs, but also led to an increase in PKG1 expression.

Silencing PKG1 mitigated fibrosis in gp120-challenged VSMCs

To confirm the function of PKG1 in fibrotic VSMCs, sh-PKG1-1 and sh-PKG1-2 were transfected into VSMCs. The results from the qPCR and WB analyses revealed that PKG1 expression was dramatically decreased by sh-PKG1-1 or sh-PKG1-2 transfection. Notably, sh-PKG1-1 more substantially decreased the PKG1 levels than did sh-PKG1-2. Given its superior silencing effects, sh-PKG1-1 was chosen for further investigation (Fig. 3A–C). Next, the VSMCs transfected with sh-PKG1 were challenged with gp120. As showed in Fig. 3D–F, sh-PKG1 notably weakened gp120-induced PKG1 expression in VSMCs. Moreover, inhibiting PKG1 decreased the viability and migration of gp120-treated VSMCs (Fig. 3G–K). In addition, inhibiting PKG1 strongly inhibited gp120-induced fibrosis-associated marker (α-SMA, MMP2, MMP3, and TIMP1) expression in VSMCs (Fig. 3L–T). The results of the immunofluorescence staining for α-SMA revealed a similar trend (Fig. 3U). Taken together, these findings suggested that decreased PKG1 expression ameliorated fibrosis in gp120-exposed VSMCs.

Discussion

Although some related studies have shown that HIV infection contributes to disease progression and increases the difficulty of treating proctological diseases, including hemorrhoids [9, 10], limited research has been conducted on the molecular mechanisms underlying poor evolution of HIV-associated hemorrhoid. The current study revealed that fibrosis and upregulation of PKG1 expression were present in tissues from HIV-infected patients with hemorrhoids. Furthermore, PKG1 silencing ameliorated gp120-stimulated VSMC proliferation, migration, and fibrosis.

Previous studies have revealed that HIV-mediated disruption of immune responses has downstream negative effects, leading to the increased release of proapoptotic, profibrotic, and proinflammatory cytokines [27]. This, in turn, triggers vasofunctional disturbances, pulmonic fibrosis [28], hepatic fibrosis [29], cardiac fibrosis [30], and fibrosis in other organs, accelerating multiple organ damage. In the present study, for the first time, evidence that fibrosis was present in tissues from HIV-positive patients with hemorrhoids was obtained. A previous study indicated that transactivator of transcription (TAT), another HIV protein, promoted proliferation in human pulmonary artery smooth muscle cells [31]. Similarly, the administration of HIV gp120 facilitated the proliferation, migration, and fibrosis of VSMCs. This evidence suggests that HIV-induced fibrosis plays an important role in the progression of hemorrhoids in HIV-positive patients. Given the complexities of the wound repair process, it is unsurprising that multiple cell types are involved in the biology of wound healing. The wound repair process is associated with fibrosis in VSMCs. Similar cellular responses have been observed in a variety of other pathological conditions related to fibrogenesis and organ remodeling. Specifically, excessive proliferation, migration, and fibrosis of VSMCs lead to increased neointimal hyperplasia and vessel wall thickness, which, in turn, results in vascular stenosis [32, 33]. This impairs vascular function, particularly the transport of oxygen and nutrients in damaged vessels. Consequently, VSMC fibrosis adversely affects wound healing process and might contribute to extended wound healing time of hemorrhoids in HIV patients. Furthermore, due to the immune deficiency of HIV-positive individuals, the postoperative wound of hemorrhoids in HIV patients is more susceptible to be infected by bacteria and viruses (including the patient's own HIV) from the rectum, perianal region, or the air environment. When it happened, wounds can be a nidus for infection, which can cause the risk of postoperative infection of these patients increase.

A recent study revealed that the treatment of human mucosal epithelial cells with HIV-1 viral particles or the gp120 protein led to the activation of PKG1 and thereby disrupted epithelial cell junctions [24]; this study provided new insight for preventing HIV transmission. Consistently, gp120 promoted PKG1 expression in VSMCs. Moreover, PKG1 was significantly increased in HIV-positive patients with hemorrhoids. Functionally, PKG1 promoted fibrosis in HIV-exposed VSMCs. This can be explained as follows: silencing PKG1 contributes to decreases in the expression of proliferation, migration, and fibrosis markers (α-SMA, MMP2, MMP3, and TIMP1). Nevertheless, other studies have shown that PKG1 has the opposite effect on fibrosis. Specifically, the silencing of the PKG1 gene makes VSMCs from young rats prone to profibrotic effects [34]. This contradictory phenomenon may be attributed to different pathological conditions and different disease backgrounds, indicating that understanding the regulatory role of PKG1 in fibrosis requires specific analyses under different conditions. In the current study, our cell experiments focused solely on VSMCs in the investigation of the role of PKG1 in HIV-induced fibrosis *in vitro*. However, HIV-induced fibrosis progression involves not only VSMCs but also other cells, such as fibroblasts, mesenchymal stem cells, and vascular endothelial cells. Therefore, the *in vivo* environment is more complex, with a wider variety of cell types involved, suggesting that the expression of a specific

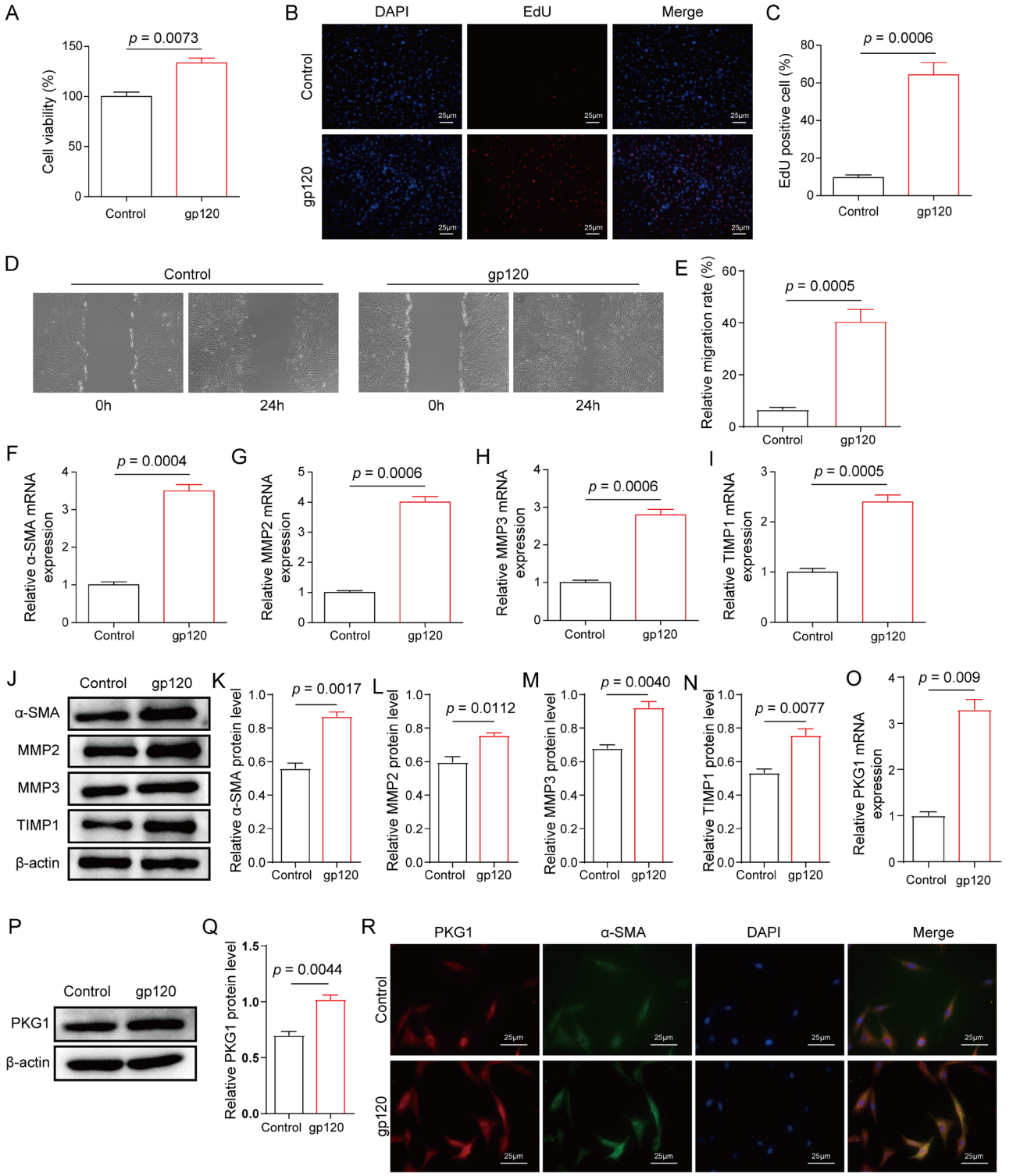

Fig. 2 Fibrosis promoted gp120-exposed VSMCs. The VSMCs were stimulated with gp120 (100 ng/mL) for 24 h to establish the cell model. **A** The results of the CCK-8 assay indicated that cell viability was increased in gp120-exposed VSMCs. **B**–**C** EdU staining revealed that cell proliferation was increased in gp120-exposed VSMCs. **D**–**E** Detection of cell migration by wound healing assay revealed that migration was increased in gp120-treated VSMCs. **F**–**I** The mRNA expressions of α-SMA, MMP2, MMP3, and TIMP1 were increased in gp120-exposed VSMCs. **J**–**N** WB analysis showed that the protein levels of α-SMA, MMP2, MMP3, and TIMP1 were enhanced in gp120-exposed VSMCs. **O** PKG1 mRNA expression was improved in gp120-exposed VSMCs. **P**–**Q** WB analysis showed that the protein level of PKG1 was improved in gp120-exposed VSMCs. **R** The upregulation of PKG1 and α-SMA in gp120-induced VSMCs was observed by immunofluorescence staining

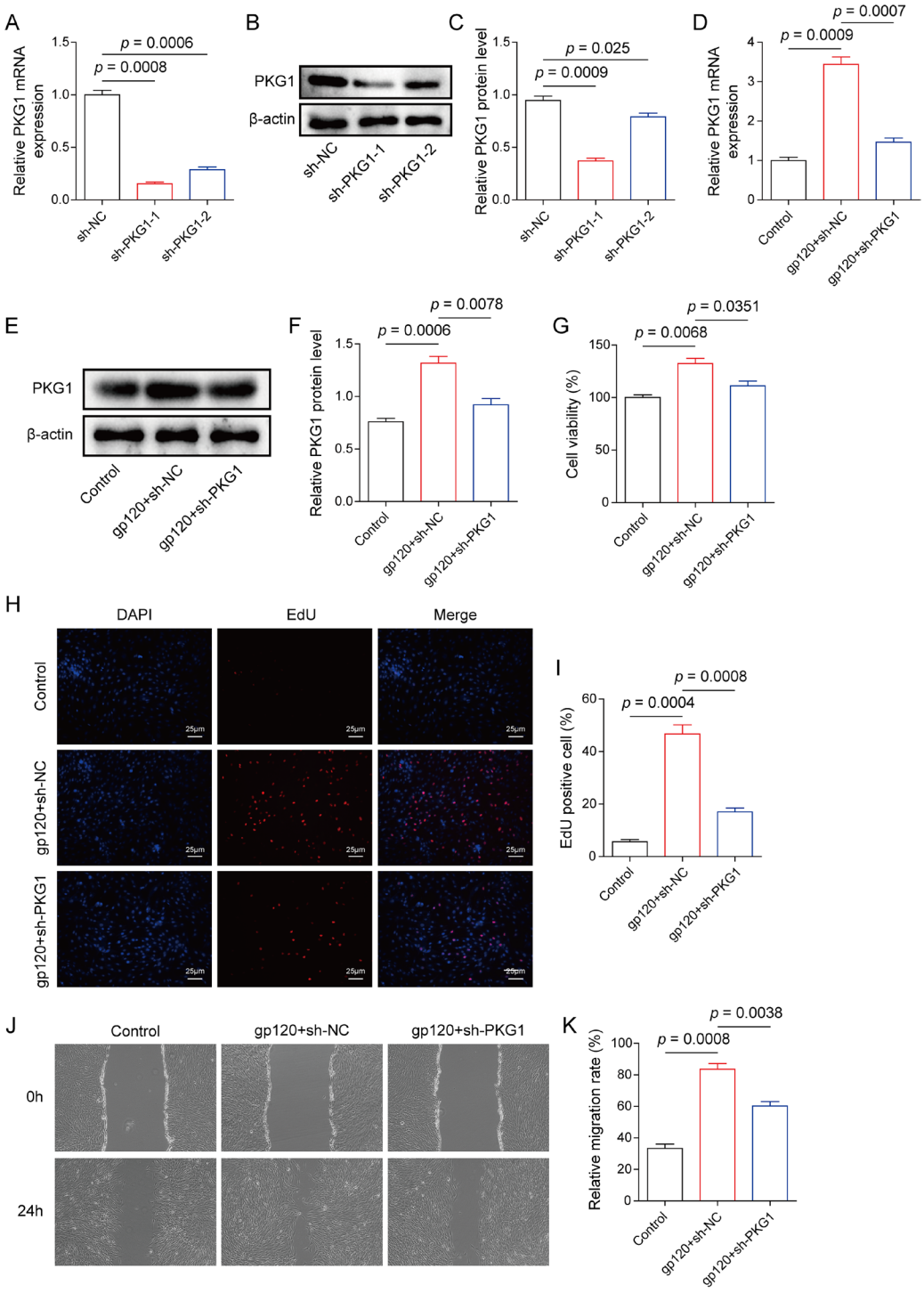

Fig. 3 Silencing PKG1 mitigated fibrosis in gp120-treated VSMCs. **A–C** VSMCs were transfected with sh-NC, sh-PKG1-1, or sh-PKG1-2. The results from qPCR and WB analyses revealed that PKG1 expression was dramatically decreased by sh-PKG1-1 or sh-PKG1-2 transfection. **C–T** VSMCs transfected with sh-PKG1 were stimulated with gp120 (100 ng/mL) for 24 h. **D–E** Transfection with sh-PKG1 notably weakened gp120-induced increases in both the mRNA (**D**) and protein (**E–F**) expression of PKG1 in VSMCs. G CCK-8 assay revealed that silencing PKG1 inhibited the viability of gp120-treated VSMCs. **H–I** EdU staining showed that silencing PKG1 strongly depressed the proliferation of gp120-treated VSMCs. **J–K** Wound healing assay showed that inhibiting PKG1 suppressed the migration of gp120-treated VSMCs. **L–O** Silencing PKG1 led to inhibit the mRNA expression of α-SMA, MMP2, MMP3, and TIMP1 in gp120-treated VSMCs. **P–T** Silencing PKG1 led to inhibit the protein levels of α-SMA, MMP2, MMP3, and TIMP1 in gp120-treated VSMCs. **U** α-SMA was detected via immunofluorescence staining. The results showed that inhibiting PKG1 suppressed gp120-induced α-SMA expression

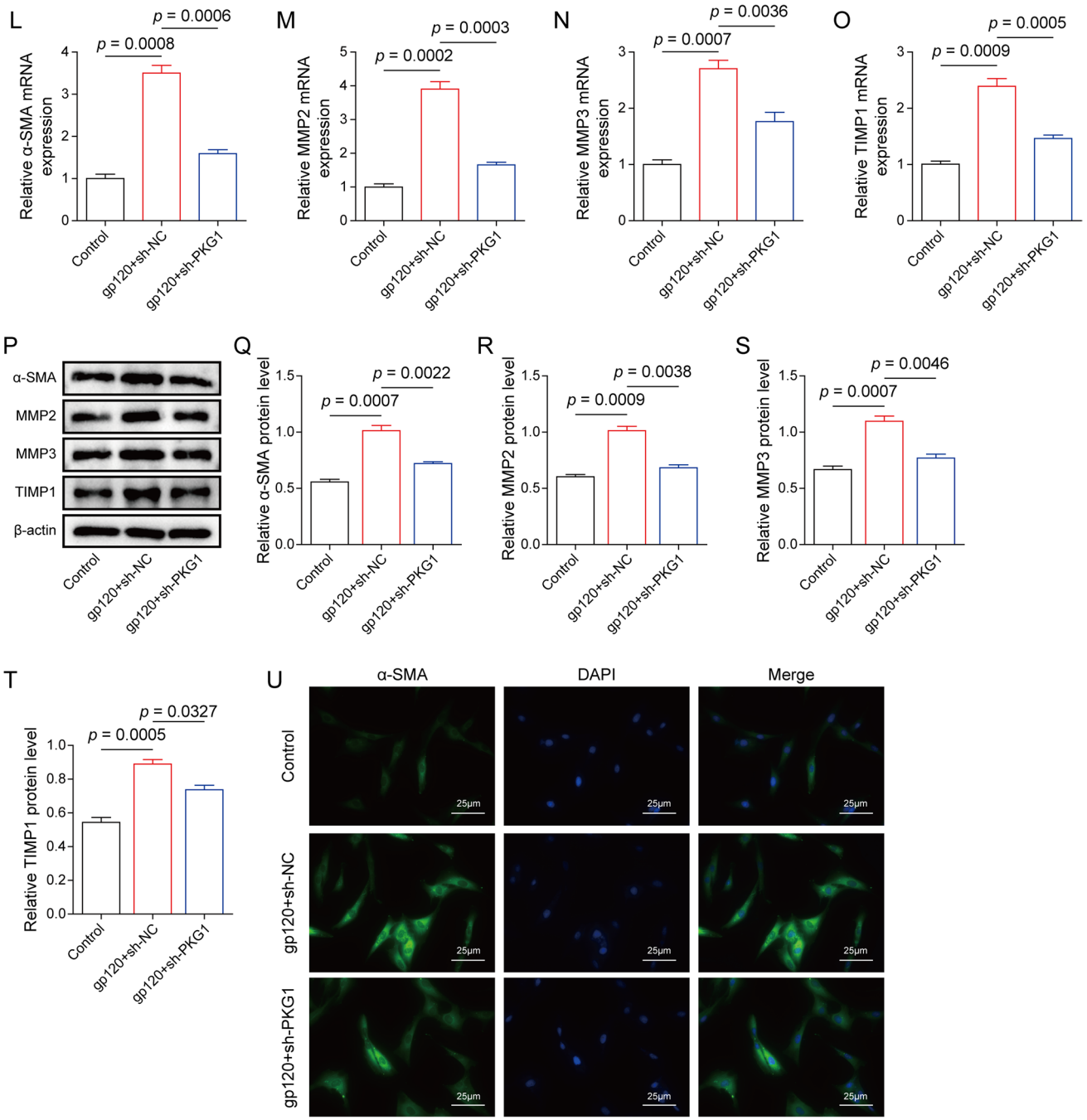

Fig. 3 (continued)

gene may not necessarily be consistent between *in vitro* and *in vivo* models. Thus, the findings might not be generalizable to other diseases or tissues.

In addition, this study relied primarily on *in vitro* experiments and cellular models, which may not fully replicate the complex interactions and dynamics that occur *in vivo*. Moreover, the present research did not explore the roles of other potential molecular mechanisms or certain downstream signaling pathways regulated by PKG1 in the development of HIV-associated hemorrhoids, which is a limitation of this study. It is possible that additional factors beyond PKG1 could contribute to the observed deleterious effects. In future studies, we will further investigate the effects of PKG1-related signaling pathways and whether they are involved in the process of HIV-induced fibrosis. A review of numerous studies revealed that the Smad2/3 signaling pathway is involved in fibrosis induced by multiple factors in various organs, tissues, and cells [35–37].

Thus, we will explore the role of the Smad2/3 signaling pathway in HIV-induced fibrosis of VSMCs. Given that PKG1 is stimulated by gp120 and contributes to the fibrosis of VSMCs, we hypothesized that PKG1 may be a promising candidate target for further investigation. For example, research on small-molecule peptides or antibodies that target PKG1 could be a promising approach. Additionally, mixed lineage kinase 3 (MLK3) is a substrate for PKG1α, whereas inositol trisphosphate receptor-associated cGMP-kinase substrate 1 (IRAG1) is a substrate for PKG1β [38, 39]. Thus, MLK3 and IRAG1 also merit further attention. Insights into the targeting of PKG1 or related signaling pathways and substrates will enable the future development of methods for inhibiting HIV-induced fibrosis progression that function through novel mechanisms of action in the treatment of HIV-associated hemorrhoids. However, additional animal studies or clinical studies on these potential therapeutic strategies for treating HIV-associated hemorrhoids are need to provide theoretical support.

Conclusions

In conclusion, this study provides the first insights into the role of PKG1 in regulating the proliferation, migration, and fibrosis of VSMCs to exert deleterious effects on HIV-associated hemorrhoids. Inhibiting PKG1 could be a promising therapeutic approach for the treatment and management of hemorrhoids in HIV-positive patients. In addition, the exploration of fibrosis of VSMCs will provide valuable directions for our future research on hemorrhoids and other anorectal diseases.

Author contributions Conceptualization: Zhen Li, Ning Wang
Methodology: Zhen Li, Shuang Peng
Software: Chun Liu, Zhong Chen
Validation: Chun Liu
Formal analysis: Zhen Li, Shuang Peng, Ning Wang
Investigation: Zhen Li, Zhong Chen
Resources: Chun Liu, Shuang Peng
Data Curation: Shuang Peng
Writing - Original Draft: Ning Wang
Writing - Review & Editing: Ning Wang, Zhen Li, Zhong Chen
Visualization: Chun Liu, Zhong Chen
Supervision: Zhen Li
Project administration: Zhen Li
All authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval This study was approved by the Ethics Committee of the First Hospital of Changsha (No. 2022–072).

Competing interests The authors declare no competing interests.

References

1. Pines H, Wertheim J, Liu L, Garfein R, Little S, Karris MJA (2016) Concurrency and HIV transmission network characteristics among MSM with recent HIV infection. Aids 30(18):2875–83. https://doi.org/10.1097/qad.0000000000001256
2. Solomon S, Solomon S, McFall A, Srikrishnan A, Anand S, Verma V et al (2019) Integrated HIV testing, prevention, and treatment intervention for key populations in India: a cluster-randomised trial. Lancet HIV 6(5):e283–e96. https://doi.org/10.1016/s2352-3018(19)30034-7
3. Zhou J, Yang L, Ma J, Jiang S, Liu Y, Sun Z (2022) Factors associated with HIV testing among MSM in Guilin, China: results from a cross-sectional study. Int J Public Health 67:1604612. https://doi.org/10.3389/ijph.2022.1604612
4. Arias Garcia S, Chen J, Calleja J, Sabin K, Ogbuanu C, Lowrance D et al (2020) Availability and quality of surveillance and survey data on HIV prevalence among sex workers, men who have sex with men, people who inject drugs, and transgender women in low- and middle-income countries: review of available data (2001–2017). JMIR Public Health Surveill 6(4):e21688. https://doi.org/10.2196/21688
5. Clifford G, Georges D, Shiels M, Engels E, Albuquerque A, Poynten I et al (2021) A meta-analysis of anal cancer incidence by risk group: toward a unified anal cancer risk scale. Int J Cancer 148(1):38–47. https://doi.org/10.1002/ijc.33185
6. de Vries H, Nori A, Kiellberg Larsen H, Kreuter A, Padovese V, Pallawela S et al (2021) 2021 European Guideline on the management of proctitis, proctocolitis and enteritis caused by sexually transmissible pathogens. J Eur Acad Dermatol Venereol 35(7):1434–43. https://doi.org/10.1111/jdv.17269
7. Nandi A, Nigar T, Das A, Dey YN, dynamics. Plumbago zeylanica (2023) Network pharmacology analysis of to identify the therapeutic targets and molecular mechanisms involved in ameliorating hemorrhoids. 1–15. https://doi.org/10.1080/07391102.2023.2280681

8. Makris G, Thulasidasan N, Malietzis G, Kontovounisios C, Saibudeen A, Uberoi R et al (2021) Catheter-directed hemorrhoidal dearterialization technique for the management of hemorrhoids: a meta-analysis of the clinical evidence. J Vasc Interv Radiol 32(8):1119–1127. https://doi.org/10.1016/j.jvir.2021.03.548
9. Moore BA, Fleshner PR (2001) Rubber band ligation for hemorrhoidal disease can be safely performed in select HIV-positive patients. Dis Colon Rectum 44(8):1079–1082. https://doi.org/10.1007/bf02234625
10. Morandi E, Merlini D, Salvaggio A, Foschi D, Trabucchi EJ (1999) Prospective study of healing time after hemorrhoidectomy: influence of HIV infection, acquired immunodeficiency syndrome, and anal wound infection. Dis Colon Rectum 42(9):1140–4. https://doi.org/10.1007/bf02238565
11. Chen B (2019) Molecular mechanism of HIV-1 entry. Trends Microbiol 27(10):878–91. https://doi.org/10.1016/j.tim.2019.06.002
12. Yu F, Jiang SJ; Biology (2022) Small-molecule HIV entry inhibitors targeting gp120 and gp41. 1366:27-43. https://doi.org/10.1007/978-981-16-8702-0_3
13. Wang P, Yang B, Huang H, Liang P, Long B, Chen L et al (2023) HIV gp120/Tat protein-induced epithelial-mesenchymal transition promotes the progression of cervical lesions. AIDS Res Ther 20(1):82. https://doi.org/10.1186/s12981-023-00577-1
14. Wallace DJP (2022) HIV-associated neurotoxicity and cognitive decline: therapeutic implications. Pharmacol Ther 234:108047. https://doi.org/10.1016/j.pharmthera.2021.108047
15. Schecter A, Berman A, Yi L, Mosoian A, McManus C, Berman J et al (2001) HIV envelope gp120 activates human arterial smooth muscle cells. Proc Natl Acad Sci 98(18):10142–7. https://doi.org/10.1073/pnas.181328798
16. Liu T, Zhou H, Lu H, Luo C, Wang Q, Peng Y et al (2021) MiR-4729 regulates TIE1 mRNA m6A modification and angiogenesis in hemorrhoids by targeting METTL14. Ann Transl Med 9(3):232. https://doi.org/10.21037/atm-20-3399
17. Yu Q, Zhao Y, Zhang X, Li W, Zhang H, Piao S et al (2024) The beneficial effect of Sanhuang ointment and its active constituents on experimental hemorrhoids in rats. J Ethnopharmacol 319:117173. https://doi.org/10.1016/j.jep.2023.117173
18. Wang C, Lu H, Luo C, Song C, Wang Q, Peng Y et al (2019) miR-412–5p targets Xpo1 to regulate angiogenesis in hemorrhoid tissue. Gene 705:167–76. https://doi.org/10.1016/j.gene.2019.04.058
19. Eugenin E, Morgello S, Klotman M, Mosoian A, Lento P, Berman J et al (2008) Human immunodeficiency virus (HIV) infects human arterial smooth muscle cells in vivo and in vitro: implications for the pathogenesis of HIV-mediated vascular disease. Am J Pathol 172(4):1100–11. https://doi.org/10.2353/ajpath.2008.070457
20. Chan M, Aminzai S, Hu T, Taran A, Li S, Kim C et al (2020) A substitution in cGMP-dependent protein kinase 1 associated with aortic disease induces an active conformation in the absence of cGMP. J Biol Chem 295(30):10394–405. https://doi.org/10.1074/jbc.RA119.010984
21. Bivalacqua T, Kendirci M, Champion H, Hellstrom W, Andersson K, Hedlund P (2007) Dysregulation of cGMP-dependent protein kinase 1 (PKG-1) impairs erectile function in diabetic rats: influence of in vivo gene therapy of PKG1alpha. BJU Int 99(6):1488–94. https://doi.org/10.1111/j.1464-410X.2007.06794.x
22. Choi S, Park M, Kim J, Park W, Kim S, Lee D et al (2018) TNF-α elicits phenotypic and functional alterations of vascular smooth muscle cells by miR-155–5p-dependent down-regulation of cGMP-dependent kinase 1. J Biol Chem 293(38):14812–22. https://doi.org/10.1074/jbc.RA118.004220
23. Di Fulvio M, Lincoln T, Lauf P, Adragna NC (2001) Protein kinase G regulates potassium chloride cotransporter-4 [corrected] expression in primary cultures of rat vascular smooth muscle cells. J Biol Chem 276(24):21046–52. https://doi.org/10.1074/jbc.M100901200
24. Xie W, Chen M, Zhai Z, Li H, Song T, Zhu Y et al (2021) HIV-1 exposure promotes PKG1-mediated phosphorylation and degradation of stathmin to increase epithelial barrier permeability. J Biol Chem 296:100644. https://doi.org/10.1016/j.jbc.2021.100644
25. Green L, Yi R, Petrusca D, Wang T, Elghouche A, Gupta S et al (2014) HIV envelope protein gp120-induced apoptosis in lung microvascular endothelial cells by concerted upregulation of EMAP II and its receptor, CXCR3. Am J Physiol-Lung Cell Mol Physiol 306(4):L372-82. https://doi.org/10.1152/ajplung.00193.2013
26. Hijmans J, Stockleman K, Reiakvam W, Levy M, Brewster L, Bammert T et al (2018) Effects of HIV-1 gp120 and tat on endothelial cell senescence and senescence-associated microRNAs. Physiol Rep 6(6):e13647. https://doi.org/10.14814/phy2.13647
27. Deeks S, Tracy R, Douek DC (2013) Systemic effects of inflammation on health during chronic HIV infection. Immunity 39(4):633–645. https://doi.org/10.1016/j.immuni.2013.10.001
28. Huang W, Tang XX (2021) Virus infection induced pulmonary fibrosis. J Transl Med 19(1):496. https://doi.org/10.1186/s12967-021-03159-9
29. Chrysanthidis T, Loli G, Metallidis S, Germanidis G (2017) Mechanisms of accelerated liver fibrosis in HIV-HCV coinfection. AIDS Rev 19(3):148–55
30. Teer E, Dominick L, Mukonowenzou N, Essop MF (2022) HIV-related myocardial fibrosis: inflammatory hypothesis and crucial role of immune cells dysregulation. Cells 11(18):2825. https://doi.org/10.3390/cells11182825
31. Guo M, Kook Y, Shannon C, Buch S (2018) Notch3/VEGF-A axis is involved in TAT-mediated proliferation of pulmonary artery smooth muscle cells: implications for HIV-associated PAH. 4:22. https://doi.org/10.1038/s41420-018-0087-9
32. Schumacher D, Liehn E, Nilcham P, Mayan D, Rattanasopa C, Anand K et al (2021) A neutralizing IL-11 antibody reduces vessel hyperplasia in a mouse carotid artery wire injury model. Sci Rep 11(1):20674. https://doi.org/10.1038/s41598-021-99880-y
33. Li G, Chen Y, Greene G, Oparil S, Thompson JA (1999) Estrogen inhibits vascular smooth muscle cell-dependent adventitial fibroblast migration in vitro. Circulation 100(15):1639–1645. https://doi.org/10.1161/01.cir.100.15.1639
34. Fedorova O, Shilova V, Zernetkina V, Juhasz O, Wei W, Lakatta E et al (2023) PKG1 silencing of gene mimics effect of aging and sensitizes rat vascular smooth muscle cells to cardiotonic steroids: impact on fibrosis and salt sensitivity. J Am Heart Assoc 12(12):e028768. https://doi.org/10.1161/jaha.122.028768
35. Zhen-Hua L, Yanhong Z, Xue W, Xiao-Fang F, Yuqing Z, Xu L et al (2019) SIRT1 activation attenuates cardiac fibrosis by endothelial-to-mesenchymal transition. 118(0). https://doi.org/10.1016/j.biopha.2019.109227
36. Zhenlin L, Ekaterina B, Ara P, Rümeyza B, Huguette L, Yuki K et al (2023) Smooth muscle α(v) integrins regulate vascular fibrosis via CD109 downregulation of TGF-β signalling. Eur Heart J Open 3(2):oead010. https://doi.org/10.1093/ehjopen/oead010
37. Hu HH, Chen DQ, Wang YN, Feng YL, Cao G, Vaziri ND et al (2018) New insights into TGF-β/Smad signaling in tissue fibrosis. 292(0). https://doi.org/10.1016/j.cbi.2018.07.008
38. Calamaras TD, Pande S, Baumgartner RAU, Kim SK, McCarthy JC, Martin GL et al (2021) MLK3 mediates impact of PKG1α on cardiac function and controls blood pressure through separate mechanisms. 6(18). https://doi.org/10.1172/jci.insight.149075
39. Biswas S, Kojonazarov B, Hadzic S, Majer M, Bajraktari G, Novoyatleva T et al (2020) IRAG1 deficient mice develop PKG1β dependent pulmonary hypertension. Cells 9(10):2280. https://doi.org/10.3390/cells9102280