

# Angiogenic function of astragaloside IV in rats with myocardial infarction occurs via the PKD1-HDAC5-VEGF pathway

LEI YANG<sup>1,2</sup>, NUAN LIU<sup>1,2</sup>, WEI ZHAO<sup>1,2</sup>, XING LI<sup>1,2</sup>, LI HAN<sup>1,2</sup>, ZHONGMING ZHANG<sup>1,2</sup>,  
YANKE WANG<sup>1</sup> and BINGYU MAO<sup>1,2</sup>

<sup>1</sup>Henan Key Laboratory of Zhang ZhongJing Formulae and Herbs for Immunoregulation;

<sup>2</sup>The Zhang ZhongJing School of Chinese Medicine, Nanyang Institute of Technology, Nanyang, Henan 473004, P.R. China

Received May 10, 2018; Accepted December 13, 2018

DOI: 10.3892/etm.2019.7273

**Abstract.** The current study aimed to assess the role and mechanism of astragaloside IV (AS-IV) in myocardial infarction. A myocardial infarction model was established via the ligation of the left anterior descending artery. Rats were randomly divided into sham, DMSO, model, AS-IV, AS-IV-CID755673 and CID755673 inhibitor groups. Rats were then sacrificed following 4 weeks of treatment and segmental heart samples were obtained for hematoxylin and eosin, and masson staining. The expression of PKD1, HDAC5 and VEGF were analyzed using immunohistochemistry, reverse transcription polymerase chain reaction and western blotting. Compared with the sham and DMSO groups, the morphology of myocardium in the model and CID755673 inhibitor groups were disordered and exhibited necrotic myocardial cells and collagen tissues. Following treatment with AS-IV, the morphology of the myocardium was markedly improved and the number of new blood vessels increased. However, following treatment with CID755673, the myocardial tissue of rats became disordered, with an increased number of necrotic cells and the closure of certain vessels. The expression of PKD1, HDAC5 and VEGF mRNA and protein in myocardial tissue of model group and CID755673 inhibitor group were significantly lower than the other four groups ( $P < 0.05$ ), whereas these levels in the AS-IV group were significantly higher than those in the other five groups ( $P < 0.01$ ). Additionally, the AS-IV-CID755673 group exhibited significantly higher levels of PKD1, HDAC5 and VEGF mRNA and protein than the sham, DMSO, CID755673 inhibitor and model groups ( $P < 0.05$ ). Furthermore, the protein expression of pS205 PKD1,

pS259 HDAC5 and pTyr951 VEGF in the myocardium of rats was comparable with that of PKD1, HDAC5 and VEGF. AS-IV may partly promote the angiogenesis of myocardial tissue in rats with myocardial infarction via the PKD1-HDAC5-VEGF pathway.

## Introduction

Myocardial infarction is characterized by regional myocardial ischemia and hypoxia as a result of artery occlusion (1) and seriously endangers patient health. Treating myocardial infarction by promoting angiogenesis has attracted increasing attention (2,3). Angiogenesis refers to the process of neovascularization in existing blood vessels, which involves the massive growth, migration, differentiation and lumen formation of vascular endothelial cells (4). Protein kinase D1 (PKD1), also known as protein kinase  $C\mu$ , belongs to the serine/threonine protein kinase family (5). However, PKD1 differs from the PKC family in structure enzymology and other regulatory properties (6-8). A previous study revealed that PKD1 exhibited a significant angiogenic effect *in vitro* and *in vivo*, and this effect was closely associated with the upregulation of vascular endothelial growth factor (VEGF) and its receptor, fins-like tyrosine kinase-1 expression (8). One of the direct downstream target proteins of PKD1 is class IIa histone deacetylase 5 (HDAC5) (9), which serves an important role in the induction of chromatin modification and the regulation of gene expression. PKD1 may bind to HDAC5, upregulating HDAC5-regulated gene expression and thus regulating vascular endothelial cell migration and proliferation (10).

Previous studies have determined the critical roles of PKD1 and HDAC5 in VEGF-induced gene expression and angiogenesis (9,11). It was also revealed that VEGF stimulates HDAC5 phosphorylation and nuclear export in endothelial cells via the VEGF receptor 2-phospholipase C-protein kinase C-PKD-dependent pathway (11). Additionally, the PKD-HDAC5 pathway mediates the transcriptional activation of myocyte enhancer factor-2 and the expression of a specific subset of genes in response to VEGF, including nuclear receptor subfamily 4 group A (NR4A1), an orphan nuclear receptor involved in angiogenesis (11). Furthermore, a HDAC5 mutant was demonstrated to inhibit VEGF-mediated NR4A1

---

*Correspondence to:* Professor Bingyu Mao, Henan Key Laboratory of Zhang ZhongJing Formulae and Herbs for Immunoregulation, Nanyang Institute of Technology, 80 Changjiang Road, Nanyang, Henan 473004, P.R. China  
E-mail: maobingyu2005@126.com; bingyumao2014@126.com

**Key words:** astragaloside IV, protein kinase D1, vascular endothelial growth factor, myocardial infarction, class II histone deacetylase 5, angiogenesis

expression, endothelial cell migration and *in vitro* angiogenesis (11). These results indicate that the PKD-HDAC5 pathway serves an important role in VEGF transcriptional regulation and angiogenesis (10,11).

CID755673 is a specific blocker of PKD1 that was utilized in the current study. Unlike many other kinase inhibitors, CID755673 contains the kinase ATP-binding domain (12), indicating its high specificity. It inhibits PKD-regulated processes, including class IIa HDAC phosphorylation (13) and has been used to inhibit prostate cancer growth and motility (12), and pancreatitis *in vivo* (14) in a PKD-dependent manner.

Previous studies have also revealed that astragalus exhibits an efficient angiogenic effect by regulating bone marrow-derived endothelial progenitor cells and inducing novel angiogenesis in myocardial tissue of myocardial infarction rats (15,16). Astragaloside IV (AS-IV; chemical formula, C<sub>41</sub>H<sub>68</sub>O<sub>14</sub>) is one of the most important components of astragalus. AS-IV is primarily used for the prevention and treatment of cardiovascular diseases, cerebrovascular diseases, immune disorders, pulmonary fibrosis, liver cancer, diabetes, kidney disease and for reducing aging (17). AS-IV inhibits platelet aggregation and causes an increase in prostacyclin and nitric oxide, thereby exerting its anti-thrombotic effect (15). It has been proposed that AS-IV may increase microvessel density in the ischemic heart of rats (17). It has been demonstrated that AS-IV may stimulate angiogenesis of human umbilical vein endothelial cells, which is accompanied by the deposition of the hypoxia-inducible factor-1 $\alpha$  protein and VEGF gene transcription (18). However, little is known about the role of AS-IV in PKD1-HDAC5-VEGF signaling. The current study assessed whether AS-IV induces angiogenesis by regulating the PKD1-HDAC5-VEGF signaling pathway in rats with myocardial infarction.

## Materials and methods

**Animals, instruments and reagents.** A total of 48 male Sprague Dawley (SD) rats (specific pathogen free grade; age, 8 weeks; weight, 200 $\pm$ 20 g) were purchased from Henan Experimental Animal Center [Zhengzhou, China; animal production license no. SCXK (Yu) 2015-0005; animal quality certificate no. 1001297]. Rats were housed in individual ventilated cages at a temperature of 22-26°C and a humidity of 53-60% under a 12 h light/dark cycle, with ambient noise <45 dB. Animals had *ad libitum* access to food and water. All animal experiments were performed in accordance with the ethical guidelines of the Animal Care Committee of Nanyang Institute of Technology and was approved by the Ethics Committee of Nanyang Institute of Technology (approval no. NYISTAEEC-2017026).

**Myocardial infarction model.** A myocardial infarction model was established as described previously (8). SD rats were anesthetized via an intraperitoneal injection of sodium pentobarbital (30 mg/kg). The left anterior descending coronary artery was then exposed and ligated for 30 min. The respective artery of the sham group was only threaded, not ligated. Penicillin (800,000 units) was injected intraperitoneally for three days to prevent infection.

**Animal grouping and treatment.** Rats in the sham and DMSO groups (each, n=8) were randomly selected prior to surgery. Rats that survived >48 h following surgery were randomly divided into a model group, an AS-IV treatment group, an AS-IV-CID755673 group and a CID755673 inhibitor group (each, n=8). Rats in AS-IV group received 40 mg/kg/d AS-IV (dissolved in 1% DMSO; cat. no. 84687434; high performance liquid chromatography >98%; Shanghai Baoman Biotechnology Co., Ltd., Shanghai, China) 72 h following surgery. Rats in the CID755673 inhibitor group and the AS-IV-CID755673 group were administered 10 mg/kg/d CID755673 (dissolved in saline; MedChemExpress, Monmouth Junction, NJ, USA; cat. no. 2011756) 48 h following surgery. Rats in the AS-IV-CID755673 group were also administered 40 mg/kg/d AS-IV 72 h following surgery. The sham and model groups received 1 ml normal saline, while those in the DMSO group were administered the same quantity of normal saline with 1% DMSO. All administrations were performed via a tail vein injection every other day. Following 4 weeks, anesthesia was administered to all rats via an intraperitoneal injection of 50 mg/kg sodium for 30 min. Rats were then sacrificed via carotid arterial exsanguination. The heart apex was collected for histological analysis, reverse transcription-polymerase chain reaction or western blot analysis. In total, 2 rats in the model group, 1 rat in the CID755673 inhibitor group and 1 rat in the AS-IV-CID755673 group succumbed following surgery.

**Hematoxylin and eosin (H&E) staining.** Myocardial tissue from the heart apex of rats was fixed in 10% paraformaldehyde for 12 h at room temperature. H&E staining was subsequently performed according to routine procedure. Tissues were sliced into 3-4  $\mu$ m sections. Following a wash with deionized water for 1-2 min, tissue sections were stained with hematoxylin for 50 sec at room temperature and rinsed with deionized water for 1-2 min. Following a wash in absolute ethanol (containing 1% HCl), sections were subsequently stained with eosin for 6-8 sec at room temperature. A total of 5 different fields of view were analyzed under a light microscope at a magnification of x200 (Nikon Tis fluorescence microscopy; Nikon Corporation, Tokyo, Japan).

**Masson staining.** Following dewaxing and dehydration, sections of myocardial tissue were incubated with 1% hydrochloric acid solution for 3-5 sec at room temperature. After rinsing, sections were stained with a mild alkali fuchsin solution (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China) for 3 min at room temperature. After further rinsing with deionized water, samples were treated with 1% solution of phosphomolybdic acid for 1 min. Finally, sections were stained with 2% aniline blue solution (Beijing Solarbio Science and Technology Co., Ltd.) for 2 min at room temperature, followed by dehydration with 95% ethanol and embedment. Following staining, myocardial collagen fibers were stained a bluish-green and the myocardium appeared red in color under light microscope at a magnification of x400.

**RT-PCR.** Total RNA was extracted from myocardial tissue via the Trizol method (Tiangen Biotech Co., Ltd., Beijing, China). The RNA of each sample was then reverse transcribed into cDNA using Takara RNA PCR Kit (AMV) Ver.3.0 (Takara

Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's protocol. The upstream and downstream primers of PKD1, HDAC5, VEGF and  $\beta$ -actin are presented in Table I. The thermocycling conditions for PCR were as follows: 94°C for 5 min, 30 cycles of 95°C for 1 min, 45°C for 1 min and 72°C for 1 min. The reaction product (2  $\mu$ g) was then electrophoresed using 1% agarose gel, stained with ethidium bromide and visualized using UV. The relative gray scale ratio of PKD1, HDAC5, VEGF and  $\beta$ -actin was analyzed using Image Lab™ software version 2.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

**Immunohistochemical staining.** Sections of myocardial tissue were dewaxed and rehydrated in a graded alcohol series. Sections were then incubated with 0.3% hydrogen peroxide at room temperature for 6 min to inactivate endogenous peroxidase activity. Antigen retrieval was achieved by heating the specimens for 2 min at 100°C in citric acid buffer (0.01 mol/l; pH 6.0). After rinsing with PBS buffer (pH 7.2), primary antibodies against PKD1 (1:200; cat. no. AF6443; Affinity Biosciences, Cincinnati, OH, USA), HDAC5 (1:50; cat. no. AB33401; AbSci, Baltimore, MD, USA) and VEGF (1:200; cat. no. AF5131; Affinity Biosciences) were added and incubated at 4°C for 12 h. Following further rinsing, goat anti-rabbit horseradish peroxidase-conjugated immunoglobulin G secondary antibodies (1:1,000; cat. no. ab6721; Abcam, Cambridge, MA, USA) was added and incubated at room temperature for 15 min. Sample were subsequently incubated with DAB solution at room temperature for 6 min for color development. A total of 5 different fields of view were randomly selected under a light microscope with magnification of x400. The average absorbance of PKD1, HDAC5 and VEGF protein was analyzed using the NIS-Elements Software BR analysis system version 4.10.00 (Nikon Corporation).

**Western blotting.** Total protein was extracted from myocardial tissue with RIPA buffer (cat. no. R0278; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and the protein concentration was determined using the Bradford method. Extracted protein (20  $\mu$ g) was electrophoresed on 12% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. Membranes were subsequently blocked with 5% non-fat milk for 2 h at room temperature and labeled with  $\beta$ -actin (1:3,000; cat. no. AF7018; Affinity Biosciences), PKD1 (1:1,000), phosphorylated (p)S205 PKD1 (1:1,000; cat. no. 12,323-1; AbSci), HDAC5 (1:500), pS259 HDAC5 (1:500; AbSci; cat. no. 12226-1), VEGF (1:1,000) or pVEGF receptor 2 (pTyr951; 1:1,000; cat. no. MA5-15149; Thermo Fisher Scientific, Inc., Waltham, MA, USA) antibodies overnight at 4°C. Following washing, horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G secondary antibodies (1:2,000) were added and incubated for 1 h at room temperature. Membranes were developed with enhanced chemiluminescence reagent (cat. no. 170-3326) and exposed to X-ray film (both Bio-Rad Laboratories, Inc., Hercules, CA, USA) in a darkroom. The relative content of protein was calculated as the ratio of gray scale of each protein to that of  $\beta$ -actin or the ratio of the phosphorylated protein to total protein with Image Lab™ software version 2.0 (Bio-Rad Laboratories, Inc.).

Table I. Primer sequences for polymerase chain reaction.

Name	Primer (5'-3')	Product length (bp)
PKD1	F: GCATGAGCTAGCCTACAGCC	198
	R: CTAATCACGACGCTGGGACT	
HDAC5	F: AGTCTCCGCTGGGTTTGTAT	208
	R: GCATTGACGCTGGGCTTTT	
VEGF	F: CGAGACGCAGCGACAAGGCA	586
	R: ACCTCTCCAAACCGTTGGAG	
$\beta$ -actin	F: GTAAAGACCTCTATGCCAACA	142
	R: GGACTCATCGTACTCCTGCTG	

PKD1, protein kinase D1; HDAC5, class IIa histone deacetylase 5; VEGF, vascular endothelial growth factor; F, forward; R, reverse.

**Statistics analysis.** Data were processed using SPSS 16.0 statistical software (SPSS, Inc., Chicago, IL, USA) and were presented as the mean  $\pm$  standard deviation. One-way analysis of variance was performed for comparison among groups followed by Bonferroni's post-hoc test.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Morphological changes of myocardial tissues.** To assess the effect of AS-IV on the morphology of myocardial tissues, H&E staining was performed. As presented in Fig. 1, cardiomyocytes of the sham and DMSO groups were intact with uniform distribution and an orderly arrangement. A clear cellular outline was also observed, with distinct margins in the nucleus and cytoplasm. Blood vessels were also intact, with erythrocytes in blood vessel cavities. Compared with the sham and DMSO groups, myocardial cells in the model and CID755673 groups were lighter in color. Myocardial tissues were also disordered, with some of the cardiomyocytes being disrupted or out of shape. Nuclear lysis was present with fibroblasts (yellow arrows) and infarcted myocardium were replaced with collagen fibers (green arrows). Compared with the model group, the myocardial tissue in the AS-IV treatment group was arranged neatly and evenly. The overall color of cells was deeper. The proportion of fibroblasts decreased significantly and the vascular lumen was intact. Cardiomyocytes of the AS-IV-CID755673 treated group were lighter in color. Some cardiomyocytes were disordered and blurred with unclear nuclear cytoplasm (yellow arrows). These data indicate that AS-IV protects cardiomyocytes from infarction injury.

Masson staining was then performed to assess the effect of AS-IV on collagen. As presented in Fig. 2, red myocardial tissues in sham and DMSO groups were regular and clear (green arrows). Blood vessels were clear with a small quantity of blue collagen surrounding them (red arrows). However, the red myocardial tissue of the model and CID755673 group markedly decreased and appeared disorganized. Necrotic myocardial tissue was also replaced by blue collagen fibers (red arrows). Samples of these groups also exhibited disorganized



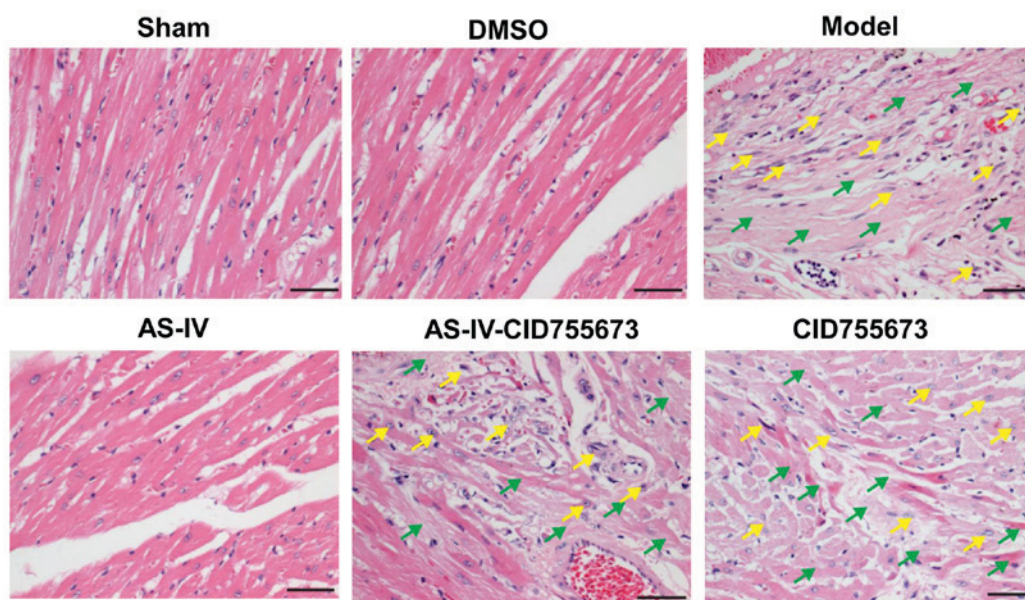


Figure 1. Effect of AS-IV on the myocardial pathomorphology in rats with myocardial infarction. H&E staining was performed to observe morphological changes. Green arrows represent collagen fibers and yellow arrows represent fibroblasts. Magnification, x400; scale bar, 50  $\mu$ m. AS-IV, astragaloside IV.

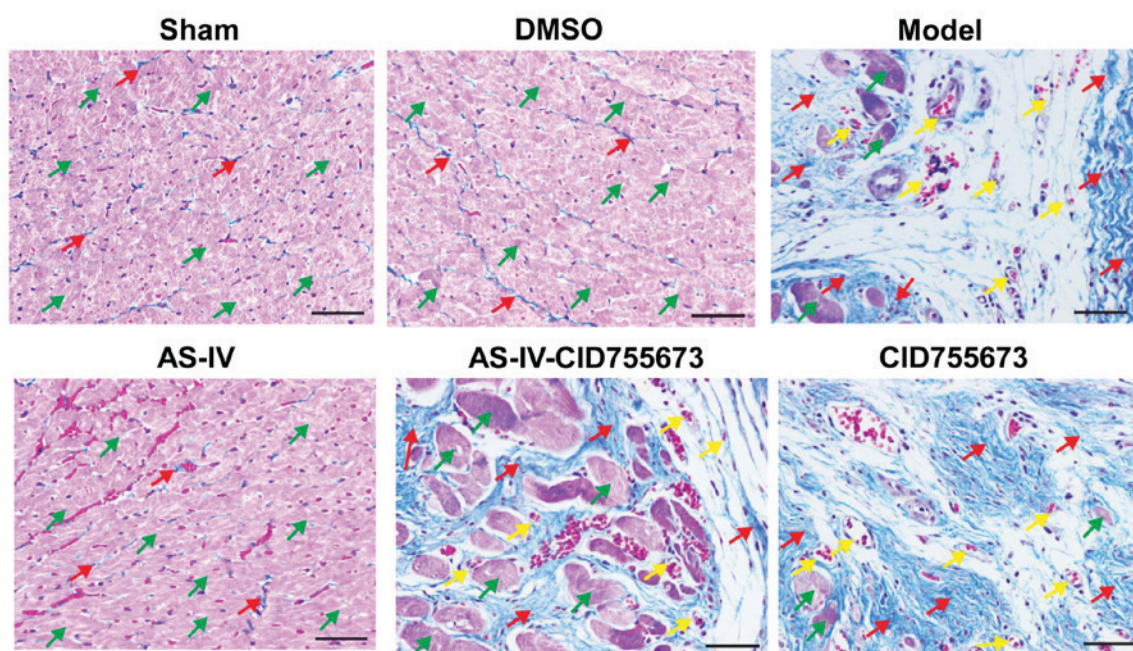


Figure 2. Effect of AS-IV on collagen fibers in rats with myocardial infarction. Masson staining was performed to observe morphological changes. Green arrows represent the red myocardial tissue; yellow arrows represent nascent granulation tissue and red arrows represent the blue collagen fibers. Magnification, x400; scale bar, 50  $\mu$ m. AS-IV, astragaloside IV.

granulation tissue, broken blood vessels and the stenosis or closure of vessel lumen. Compared with the model group, the number of blue collagen fibers in the AS-IV treatment group was lower while the number of myocardial tissues was higher (green arrows). Following the addition of CID755673, the arrangement of myocardial tissue in the AS-IV-CID755673 group became disordered, with some tissues being broken. Furthermore, the proportion of red myocardial tissue was markedly reduced and more blue collagen was observed (red arrows). Blood vessel morphology was incomplete, with stenosis and more nascent granulation tissue (yellow arrows).

These results indicate that AS-IV reduces the myocardial fibrosis of cardiac tissue.

*The effect of AS-IV on the mRNA expression of PKD1, HDAC5 and VEGF.* As determined using RT-PCR, the mRNA expression of PKD1, HDAC5 and VEGF in the myocardium of all groups were significantly decreased when compared with the AS-IV group ( $P < 0.05$ ; Fig. 3). Following the addition of CID755673, the mRNA expression of PKD1, HDAC5 and VEGF in the AS-IV-CID755673 group was significantly lower compared with the AS-IV group ( $P < 0.01$ ). However, expression

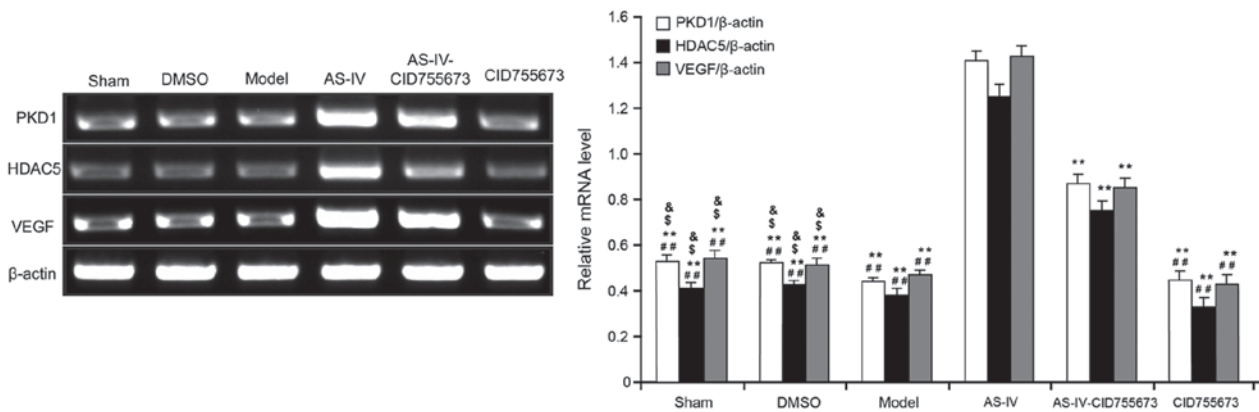


Figure 3. Effect of AS-IV on the mRNA and protein expression of PKD1, HDAC5 and VEGF in the rats with myocardial infarction. reverse-transcription polymerase chain reaction was utilized to detect mRNA expression. Data are expressed as the mean  $\pm$  standard deviation (n=6-8). \*\*P<0.01 vs. the AS-IV group; ##P<0.01 vs. the AS-IV-CID755673 group; \*P<0.05 vs. the Model group; &P<0.05 vs. the CID755673 group. AS-IV, astragaloside IV; PKD1, protein kinase D1; HDAC5, class IIa histone deacetylase 5; VEGF, vascular endothelial growth factor.

was significantly lower in all remaining groups (excluding AS-IV) when compared with the AS-IV-CID755673 group (P<0.05). These results indicate that AS-IV treatment upregulates the mRNA expression of PKD1, HDAC5 and VEGF following myocardial infarction.

*The effect of AS-IV on the protein expression of PKD1, HDAC5 and VEGF.* Western blotting revealed that the protein expression of PKD1, pS205 PKD1, HDAC5, pS259 HDAC5, VEGF and pTyr951 VEGF in the myocardium of all groups were significantly lower than the AS-IV (P<0.05 or P<0.01; Fig. 4). Following the addition of CID755673, the protein expression of PKD1, pS205 PKD1, HDAC5, pS259 HDAC5, VEGF and pTyr951 VEGF in the AS-IV-CID755673 group were significantly lower than that of AS-IV group (P<0.05 or P<0.01). Furthermore, expression was significantly lower in all remaining groups (excluding AS-IV) when compared with the AS-IV-CID755673 group (P<0.05 or P<0.01). These results indicate that AS-IV treatment upregulates the protein expression of PKD1, HDAC5 and VEGF and the phosphorylation status of PKD1, HDAC5 and VEGF receptor 2 following myocardial infarction.

The results of immunohistochemistry were consistent with that of western blotting. Cells that stained positive for VEGF expression appeared in the cytoplasm and were stained yellow or brown. Cells that were positive for PKD1 and HDAC5 expression were stained yellow or tan in the nucleus (Fig. 5). The results demonstrated that the expression of PKD1 (blue arrows), HDAC5 (red arrows) and VEGF (green arrows) in the sham and DMSO groups were significantly higher than those in the model group. Furthermore, all groups exhibited a significantly lower expression of PKD1, HDAC5 and VEGF when compared with the AS-IV (P<0.01). Compared with the AS-IV group, the expression of these proteins in the AS-IV-CID755673 group were significantly decreased (P<0.01); however, all groups (excluding the AS-IV group) were significantly lower compared with the AS-IV-CID755673 (P<0.05). These results indicate that AS-IV treatment protects the function of rat hearts following myocardial infarction partly via the PKD1-HDAC5-VEGF pathway.

## Discussion

Myocardial infarction causes irreversible tissue necrosis that results in the production of a large quantity of granulation tissue and collagen fibers, which seriously damages the normal physiological function of the heart (19). The results of H&E and Masson staining performed in the current study confirmed that myocardial tissues were severely damaged in myocardial infarction model rats, exhibiting a large proportion of damaged or missing blood vessels. The proportion of collagen tissue was also increased. These results are consistent with those of previous studies (8,15).

Astragalus is a classic Chinese medicine used for the treatment of myocardial infarction (15,16). It has been demonstrated to inhibit necrotic myocardium and stimulate the transformation of granulation tissue into capillaries for blood recanalization (15,20,21). It has been revealed that astragalus shortens the healing time of skin scabs (20,21). Furthermore, astragalus promotes the angiogenesis of rat myocardial tissue following myocardial infarction, thus promoting the development and maturation of new blood vessels (15). A previous study also demonstrated that the astragalus extract promoted the proliferation, migration and angiogenesis of endothelial progenitor cells (EPCs) (16). In the current study, a large quantity of granulation tissue with intact newborn capillaries were generated in the AS-IV treated group and the proportion of myocardial tissue was markedly higher than that in model group. This is consistent with previous findings (15,22-25), indicating that AS-IV promotes angiogenesis in myocardial tissue.

The results of RT-PCR, immunohistochemistry and western blotting further demonstrated that AS-IV upregulated the mRNA and protein expression of PKD1, HDAC5 and VEGF. Additionally, AS-IV was revealed to increase pS205 PKD1, pS259 HDAC5 and pTyr951 VEGF levels in myocardium. Previous literatures have reported that PKD1 regulates endothelial cell angiogenesis (8,26). The primary function of PKD1 is to regulate the proliferation and migration of endothelial cells, to promote the hypertrophy of vascular smooth muscle cells and to activate platelets (26). HDAC5 induces chromatin modification and inhibits the regulation of gene



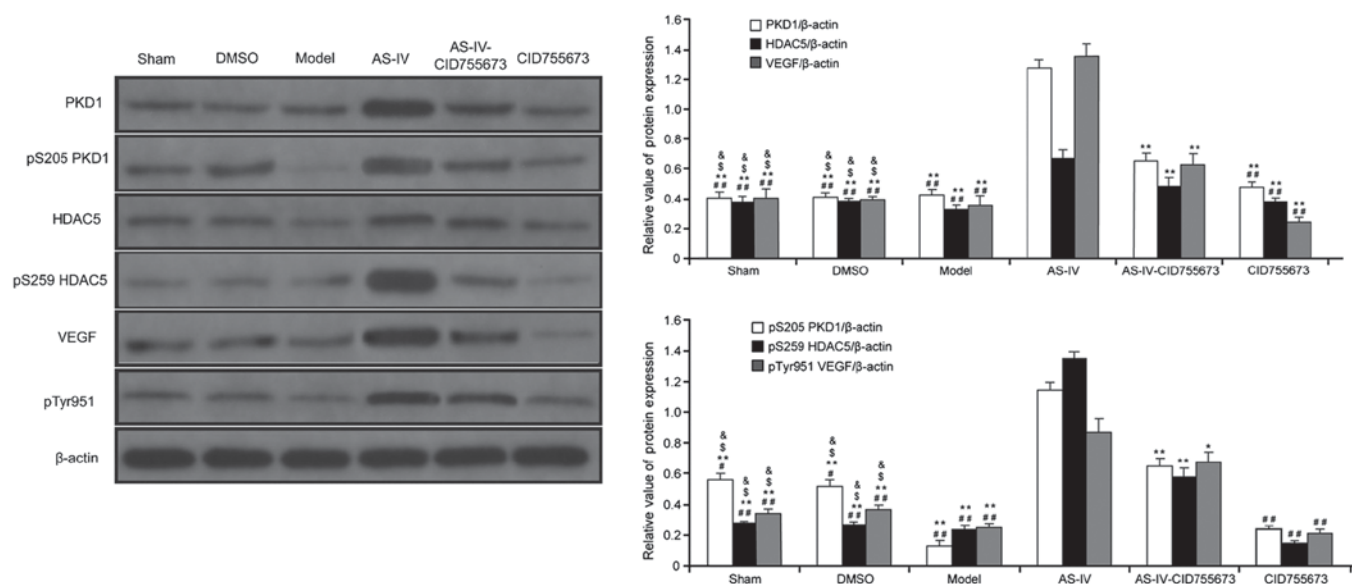


Figure 4. Effect of AS-IV on the protein expression of PKD1, pS205 PKD1, HDAC5, pS259 HDAC5, VEGF and pTyr951 VEGF in rats with myocardial infarction. Western blotting was utilized to detect protein expression. Representative and quantitative western blot results are presented. Data are presented as the mean  $\pm$  standard deviation ( $n=6-8$ ). \* $P<0.05$  and \*\* $P<0.01$  vs. the AS-IV group; # $P<0.05$  and ## $P<0.01$  vs. the AS-IV-CID755673 group; & $P<0.05$  vs. the Model group; &P<0.05 vs. the CID755673 group. AS-IV, astragaloside IV; PKD1, protein kinase D1; p, phosphorylated HDAC5, class IIa histone deacetylase 5; VEGF, vascular endothelial growth factor.

expression and is a direct downstream target protein of PKD1 in endothelial cells (10,11). The binding of PKD1 and HDAC5 may upregulate HDAC5-controlled gene expression, regulate the migration and proliferation of vascular endothelial cells, mediate signal transduction pathways in endothelial cells, and regulate VEGF-induced angiogenesis (10,11). VEGF stimulates the PKD1-dependent phosphorylation of serine 259/498 residues in the HDAC5 region of endothelial cells (27), which induces the translocation of HDAC5 from the nucleus to the cytoplasm and leads to the expression, migration and lumen formation of myocyte enhancer binding factor 2 (10,11), serving an important role in angiogenesis and the maintenance of vascular integrity (10,26).

Following CID755673 treatment in the current study, the proportion of myocardial tissue decreased and the expressions of PKD1, HDAC5 and VEGF were downregulated, compared with the AS-IV treatment group. These results indicate that CID755673 blocks the upregulation of PKD1 expression induced by AS-IV. Previous studies have revealed that PKD1 significantly promotes the adhesion, migration and proliferation of EPCs, upregulates the expression of VEGF in EPCs and promotes the proliferation and migration of endothelial cells (8,16). PKD1 also promotes the upregulation of VEGF and its receptor, kinase insert domain receptor, in myocardium, serving an important role in the repair of ischemic myocardium (8). The present study therefore hypothesizes that AS-IV may serve a role in the promotion of angiogenesis in myocardial tissue following myocardial infarction by regulating the PKD1-HDAC5-VEGF signaling pathway.

Previous studies have demonstrated that CID755673 blocks the pro-angiogenic effect of PKD1 (8,28). However, in the current study, the expression of PKD1, HDAC5 and VEGF in cardiac muscle tissue treated with CID755673 and AS-IV were significantly decreased compared with AS-IV group. Despite this, these expressions remained significantly higher than that

of model, sham, DMSO and CID755673 groups, indicating that CID755673 inhibits the pro-angiogenic effect of AS-IV partly by blocking the PKD1-HDAC5-VEGF signaling pathway. However, this effect is only partial, which may be the result of the multi-target effect of AS-IV or the complexity of inhibition mechanisms. This matter should be assessed in future studies.

CID755673 is a universal PKD inhibitor and thus it inhibits PKD2, an important subtype of PKD and an important mediator of angiogenesis (12,29). However, the current study did not detect changes in PKD2, which serves as a limitation. PKD2 levels will be assessed in future research.

In conclusion, the present study demonstrated that angiogenesis is markedly stimulated by AS-IV treatment and that the mRNA and protein accumulation of PKD1, HDAC5 and VEGF in cardiac muscle is upregulated, which could be partly inhibited by a specific inhibitor of PKD1, CID755673. These results indicate the involvement of the PKD1-HDAC5-VEGF pathway in the promotion of angiogenesis as induced by AS-IV. The current study provides novel insight into the angiogenic effects and mechanism of AS-IV and provides evidence that the PKD1-HDAC5-VEGF pathway acts as a regulator in ischemic myocardium treated with AS-IV.

#### Acknowledgements

Not applicable.

#### Funding

The current study was supported by the National Natural Science Foundation of China (grant nos. 81473438, 81873106 and 81202791), the Science and Technology Project of Henan Province (grant no. 162102310011), the Key Research Projects of Henan Colleges and Universities (grant nos. 19A360030 and 18B360011), the Funding Scheme for the Young Backbone

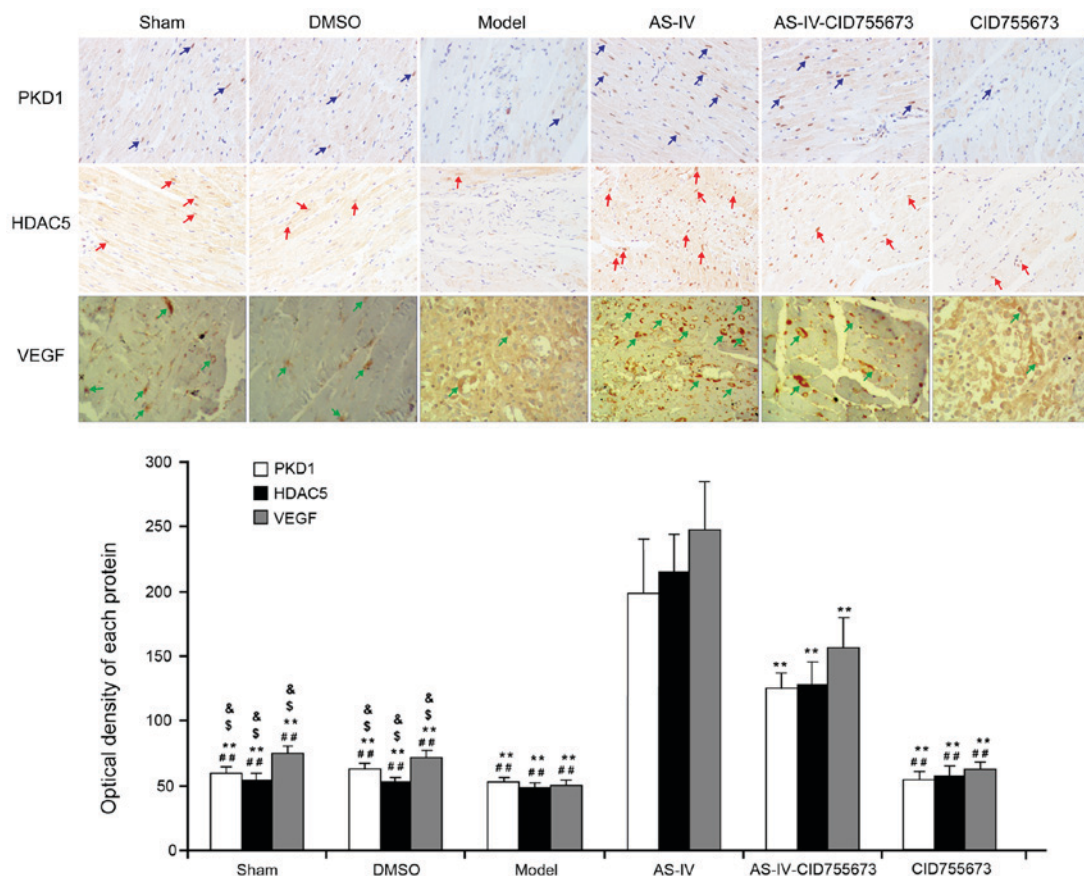


Figure 5. Effect of AS-IV on the protein expression of PKD1, HDAC5 and VEGF in rats with myocardial infarction. Immunohistochemistry was performed to detect protein expression. Representative and quantitative immunohistochemistry results are presented in the upper and lower panel. Blue arrows represent positive cells for PKD1 expression, red arrows represent the positive cells for HDAC5 expression and green arrows represent the positive cells for VEGF expression. Data are presented as the mean  $\pm$  standard deviation (n=6-8). Magnification, x400; scale bar, 50  $\mu$ m. \*\*P<0.01 vs. the AS-IV group; ##P<0.01 vs. the AS-IV-CID755673 group; \$P<0.05 vs. the Model group; &P<0.05 vs. the CID755673 group. AS-IV, astragaloside IV; PKD1, protein kinase D1; HDAC5, class IIa histone deacetylase 5; VEGF, vascular endothelial growth factor.

Teachers of Higher Education Institutions in Henan Province (grant no. 2015GGJS-255), Henan Natural Science Foundation (grant no. 162300410203) and the Science and Technology Project of Nanyang City (grant no. 2014GG043).

#### Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Authors' contributions

LY performed the experiments and wrote the manuscript. NL performed immunohistochemical staining. WZ and XL performed hematoxylin and eosin, and Masson staining. LH performed western blotting. ZZ extracted RNA and performed reverse transcription polymerase chain reaction. YW constructed the animal models. BM designed the research.

#### Ethics approval and consent to participate

All animal experiments were performed according to the ethical guidelines of Zhang Zhongjing School of Chinese Medicine. The study was also approved by this institution.

#### Patient consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

#### References

- Zuin M, Rigatelli G, Fogato L, Faggian G, Zuliani G and Roncon L: Massive myocardial infarction due to the complete occlusion of the left anterior descending coronary artery after blunt chest trauma. *Cardiovasc Revasc Med* 19: 810-811, 2018.
- Fan ZG, Qu XL, Chu P, Gao YL, Gao XF, Chen SL and Tian NL: MicroRNA-210 promotes angiogenesis in acute myocardial infarction. *Mol Med Rep* 17: 5658-5665, 2018.
- Zhou XL, Zhu RR, Liu S, Xu H, Xu X, Wu QC and Liu JC: Notch signaling promotes angiogenesis and improves cardiac function after myocardial infarction. *J Cell Biochem* 119: 7105-7112, 2018.
- Patan S: Vasculogenesis and angiogenesis. *Cancer Treat Res* 117: 3-32, 2004.
- di Blasio L, Droetto S, Norman J, Bussolino F and Primo L: Protein kinase D1 regulates VEGF-A-induced alphavbeta3 integrin trafficking and endothelial cell migration. *Traffic* 11: 1107-1118, 2010.
- Yu D, Chen W, Ren J, Zhang T, Yang K, Wu G and Liu H: VEGF-PKD1-HDAC7 signaling promotes endothelial progenitor cell migration and tube formation. *Microvasc Res* 91: 66-72, 2014.

7. Evans IM and Zachary IC: Protein kinase D in vascular biology and angiogenesis. *Iubmb Life* 63: 258-263, 2011.
8. Mao BY, Liu N, Yang L, Xu GC and Ye SS: Protein kinase D1 (PKD1) promotes angiogenesis following myocardial infarction via vascular endothelial growth factor (VEGF) pathway. *Int J Clin Exp Med* 10: 10528-10534, 2017.
9. Matthews SA, Liu P, Spitaler M, Olson EN, McKinsey TA, Cantrell DA and Scharenberg AM: Essential role for protein kinase D family kinases in the regulation of class II histone deacetylases in B lymphocytes. *Mol Cell Biol* 26: 1569-1577, 2006.
10. Ha CH and Jin ZG: Protein kinase D1, a new molecular player in VEGF signaling and angiogenesis. *Mol Cells* 28: 1-5, 2009.
11. Ha CH, Wang W, Jhun BS, Wong C, Hausser A, Pfizenmaier K, McKinsey TA, Olson EN and Jin ZG: Protein kinase D-dependent phosphorylation and nuclear export of histone deacetylase 5 mediates vascular endothelial growth factor-induced gene expression and angiogenesis. *J Biol Chem* 283: 14590-14599, 2008.
12. Venardos K, De Jong KA, Elkamie M, Connor T and McGee SL: The PKD inhibitor CID755673 enhances cardiac function in diabetic db/db mice. *PLoS One* 10: e0120934, 2015.
13. Sharlow ER, Giridhar KV, LaValle CR, Chen J, Leimgruber S, Barrett R, Bravo-Altamirano K, Wipf P, Lazo JS and Wang QJ: Potent and selective disruption of protein kinase D functionality by a benzoxoloazepinone. *J Biol Chem* 283: 33516-33526, 2008.
14. Yuan J, Liu Y, Tan T, Guha S, Gukovsky I, Gukovskaya A and Pandolfi SJ: Protein kinase d regulates cell death pathways in experimental pancreatitis. *Front Physiol* 3: 60, 2012.
15. Liu N, Yang L, Mao BY, XU GC and YE SS: Promoting angiogenesis effect of astragali radix extract on myocardium of rats with myocardial infarction. *Chin J Exp Tradit Med Formulae* 19: 92-96, 2015.
16. Liu W, Bing Y, Yang L, Xu G, Ye S, Zhang P and Zhang Y: Effect of *Astragalus membranaceus* L. extract on rat bone marrow-derived endothelial progenitor cells. *Tianjin yi yao za zhi* 10: 1093-1096, 2015.
17. Yu JM, Zhang XB, Jiang W, Wang HD and Zhang YN: Astragalosides promote angiogenesis via vascular endothelial growth factor and basic fibroblast growth factor in a rat model of myocardial infarction. *Mol Med Rep* 12: 6718-6726, 2015.
18. Zhang L, Liu Q, Lu L, Zhao X, Gao X and Wang Y: Astragaloside IV stimulates angiogenesis and increases hypoxia-inducible factor-1 $\alpha$  accumulation via phosphatidylinositol 3-kinase/Akt pathway. *J Pharmacol Exp Ther* 338: 485-491, 2011.
19. Luo KQ, Long HB and Xu BC: Reduced apoptosis after acute myocardial infarction by simvastatin. *Cell Biochem Biophys* 71: 735-740, 2014.
20. Yang Y, Chin A, Zhang L, Lu J and Wong RW: The role of traditional Chinese medicines in osteogenesis and angiogenesis. *Phytother Res* 28: 1-8, 2014.
21. Li H, Wan H, Xia T, Chen M, Zhang Y, Luo X and Li X: Therapeutic angiogenesis in ischemic muscles after local injection of fragmented fibers with loaded traditional Chinese medicine. *Nanoscale* 7: 13075-13087, 2015.
22. Wang SG, Xu Y, Chen JD, Yang CH and Chen XH: Astragaloside IV stimulates angiogenesis and increases nitric oxide accumulation via JAK2/STAT3 and ERK1/2 pathway. *Molecules* 18: 12809-12819, 2013.
23. Chen X, Peng LH, Shen YH, Li N, Wei W, Yu L, Li QM, Liang WQ and Gao JQ: Astragaloside IV-loaded nanoparticle-enriched hydrogel induces wound healing and anti-scar activity through topical deliver. *Int J Pharm* 447: 171-181, 2013.
24. Zhang L, Yang Y, Wang Y and Gao X: *Astragalus membranaceus* extract promotes neovascularisation by VEGF pathway in rat model of ischemic injury. *Pharmazie* 66: 144-150, 2011.
25. Han L, Liu N, Yang L, Mao Y and Ye SS: *Astragalus membranaceus* extract promotes angiogenesis by inducing VEGF, CD34 and eNOS expression in rats subjected to myocardial infarction. *Int J Clin Exp Med* 9: 5709-5718, 2016.
26. Evans IM, Bagherzadeh A, Charles M, Raynham T, Ireson C, Boakes A, Kelland L and Zachary IC: Characterization of the biological effects of a novel protein kinase D inhibitor in endothelial cells. *Biochem J* 429: 565-572, 2010.
27. Wang S, Li X, Parra M, Verdin E, Bassel-Duby R and Olson EN: Control of endothelial cell proliferation and migration by VEGF signaling to histone deacetylase 7. *Proc Natl Acad Sci USA* 105: 7738-7743, 2008.
28. Liu N, Yang L, Mao BY and Zhang LF: The role of protein kinase D1 in angiogenesis in rat bone marrow derived endothelial progenitor cells. *Chin J Pharmacol* 31: 1259-1263, 2015.
29. Lavalle CR, Bravo-Altamirano K, Giridhar KV, Chen J, Sharlow E, Lazo JS, Wipf P and Wang QJ: Novel protein kinase D inhibitors cause potent arrest in prostate cancer cell growth and motility. *BMC Chem Biol* 10: 5, 2010.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.