

Deletion of P110 δ promotes the development of myocarditis in ApoE-deficient mice by increasing mononuclear cell peritoneal infiltration

QI-ZHI ZHANG¹, AI-YING XUE², WEI WEI³, AI-MIN PANG¹, LI-NA CAO² and FANG LIU²

¹Out-Patient Department, ²Department of Cardiology, ³Nuclear Medicine,
The Second Hospital of Shandong University, Jinan, Shandong 250000, P.R. China

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Abstract. Phosphoinositide 3-kinase catalytic subunit δ isoform (P110 δ) is mainly expressed in white blood cells. It is involved in T and B lymphocyte differentiation, maturation and the neutrophil chemotaxis process. Apolipoprotein E (ApoE) is an arginine-rich alkaline protein, which is present in plasma chylomicron, low-density lipoprotein and very low-density lipoprotein. The present study aimed to determine the effects of P110 δ deletion on myocarditis in ApoE^{-/-} mice. A mouse model of ApoE and P110 δ double deletion was initially constructed; hematoxylin and eosin (H&E) staining was performed to detect the histological alterations in the mouse myocardium. Systolic and diastolic alterations, and alterations in the left ventricular fractional shortening (LVFS) and left ventricular ejection fraction (LVEF) were examined by electrocardiogram. Blood cell of ApoE and P110 δ double mice was used to detect changes in white blood cells and monocytes. Western blotting was used to detect the expression levels of apoptosis-associated proteins, whereas flow cytometry was used to detect the percentage of apoptosis. Morphological alterations in myocardial cells were observed under a microscope. The results of polymerase chain reaction demonstrated that double deletion mice were successfully constructed. H&E staining revealed that cells in the ApoE^{-/-} mice were spindle-shaped; however, the nuclei were smaller in the double deletion mice. There was no change in cardiac contraction in normal mice; however, in double deletion mice, the systolic and diastolic contractions were markedly reduced. LVFS and LVEF were decreased compared with in the control group. Blood cell analysis indicated that the content of white blood cells and monocytes in the experimental group was significantly higher than that in the control group. Western

blotting demonstrated that the expression levels of apoptotic proteins in double deletion mice were significantly higher compared with in the control group. Flow cytometry revealed that the apoptotic ratio was increased in double deletion mice compared with in the control group (42 vs. 21%). These findings suggested that deletion of P110 δ may induce monocyte peritoneal infiltration and increase apoptosis, thus promoting the development of myocarditis.

Introduction

Cardiac disease is currently the largest risk factor for of adult mortality and is associated with ≤ 17 million cases of mortality per year worldwide (1). Myocarditis is an inflammatory condition of the heart, which can be induced by viral infection, hypersensitivity to certain substances and autoimmune reactions (2). If it is not treated properly, myocarditis may lead to dilated cardiomyopathy, which may result in the development of heart failure. Although research in this area has advanced rapidly in recent years, the therapeutic strategies used to cure myocarditis remain limited. At present, physicians generally believe that myocarditis is the result of various pathogenic factors. The development of myocarditis is accompanied by lipid metabolism disorders, and inflammatory and immune responses (3,4).

Apolipoprotein E (ApoE) is an arginine-rich alkaline protein, which is present in plasma chylomicron, low-density lipoprotein and very low-density lipoprotein (5). ApoE can be synthesized in various tissues, mainly in the liver, brain and kidney. ApoE is a ligand for low-density lipoprotein receptors and for hepatocyte remnant receptors; therefore it is closely associated with lipoprotein metabolism. ApoE is polymorphic, and is dependent on individual lipid levels and is closely associated with the development of atherosclerosis. In addition, ApoE is involved in the activation of hydrolyzed fats, immunoregulation and regeneration of nerve tissue (6-8). However, to the best of our knowledge, the relationship between ApoE and myocarditis has not yet been investigated. Given the important role of ApoE in cardiac development, the present study aimed to determine whether ApoE is also involved in myocarditis.

Phosphoinositide 3-kinase (PI3K) is an intracellular phosphatidylinositol kinase, which is known to regulate

Correspondence to: Dr Fang Liu, Department of Cardiology, The Second Hospital of Shandong University, 247 Beiyuan Road, Jinan, Shandong 250000, P.R. China
E-mail: fangliu9898@163.com

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inflammatory responses in numerous diseases (9,10). The inhibition of PI3K is also able to promote infarct resorption and prevent adverse cardiac remodeling following myocardial infarction in mice (11). A study using PI3K γ -deficient mice demonstrated a complex contribution of PI3K γ to reparative angiogenesis in myocardial infarction (12). PI3K catalytic subunit δ isoform (P110 δ) is an enzyme that in humans is encoded by the PIK3CD gene (13), which is widely involved in cell growth, differentiation, and immune regulation and other effects. P110 δ is mainly expressed in white blood cells, and it is involved in T and B lymphocyte differentiation, maturation and the neutrophil chemotaxis process; therefore, P110 δ is considered an important molecule that regulates the leukocyte immune response (14). In addition, it has been suggested that P110 δ is an attractive pharmacological target that modulates unwanted immune responses and certain blood cancers (15). Notably, P110 δ -selective inhibitors are currently being tested in clinical trials to treat autoimmunity, allergies and lymphoid malignancies (16).

In recent years, there have been many reports about the relationship between P110 δ and inflammation. Previous studies have reported that B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax) and BH3 interacting-domain death agonist (Bid) have an important role in transforming growth factor (TGF)- β 1-induced myocarditis and apoptosis. TGF- β 1 was able to promote the expression and activation of P110 δ , and phosphorylate p21 through Bax and Bid (15). The lack of P110 δ can effectively reverse TGF- β 1-induced myocarditis (17); therefore, it may be suggested that P110 δ and myocarditis are closely related. A previous study suggested that P110 δ is able to inhibit monocyte infiltration in ApoE knockout (KO) mice (18). In present study, ApoE $^{-/-}$ /P110 $\delta^{-/-}$ mice were constructed on the basis of ApoE $^{-/-}$ mice and investigated the effects of P110 δ deletion on myocarditis in ApoE KO mice.

Materials and methods

Animal model. ApoE $^{-/-}$ mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). P110 $\delta^{-/-}$ mice were constructed by ourselves as described previously (19). Wild-type (WT) mice were purchased from the Experimental Animal Center of Guangdong Province (Guangzhou, China). The 12 mice (6 female and 6 male, weighted 20-25 g) were weaned on the 22nd day after birth and fed a normal diet and food and water was free including 4% fat and 0.07% cholesterol). The mice were maintained under a specific pathogen-free conditions with 12-h light/dark cycles at 26-28°C and 50-65% humidity. DNA was extracted from the tail tissues according to the MagBeads Tissues Gen DNA Extraction kit (D1700, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). The expression levels of ApoE and P110 δ were determined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The primers used are as follows: ApoE, sense 5'-GCCTAGCCGAGGGAGAGCCG-3', antisense 5'-TGTGACTTGGGAGCTCTGCAGC-3'; and P110 δ , sense 5'-CTGTCATCTCACCTTGCTCC-3' and antisense 5'-AGCGAACC GCCCTATGAC-3'. The reaction conditions were as follows: 94°C for 3 min, followed by 35 cycles at 94°C for 20 sec, 65°C for 30 sec and 72°C for 15 sec. Mice with ApoE/P110 δ deletion were screened from

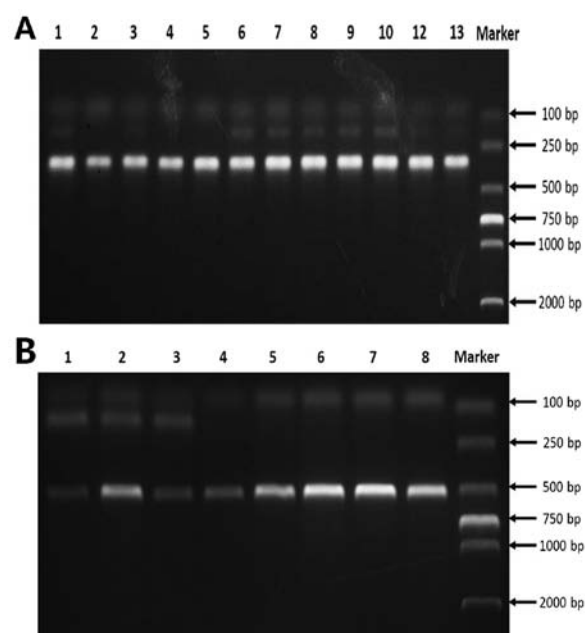


Figure 1. Construction of a mouse model. (A) Identification of ApoE $^{-/-}$ mice. ApoE $^{+/+}$ with two bands at 165 and 330 bp. Lanes 1, 6, 7, 8, 9 and 10 are ApoE $^{+/+}$ with two bands at 165 and 330 bp; Lanes 2, 3, 4, 5, 11 and 12 are ApoE $^{-/-}$ with one band at 330 bp. (B) Identification of P110 $\delta^{-/-}$ mice. P110 $\delta^{+/+}$ homozygous with one band at 485 bp. Lanes 1, 2 and 3 are P110 $\delta^{+/+}$ heterozygous with two bands at 145 and 485 bp; Lanes 4, 5, 6, 7 and 8 are P110 $\delta^{-/-}$ homozygous with one band at 485 bp. ApoE, apolipoprotein E; P110 δ , phosphoinositide 3-kinase catalytic subunit δ isoform.

the hybrid F2 generation and were used as experimental mice, whereas ApoE $^{-/-}$ mice were used as control mice. Mice in the experimental group (n=6) and control group (n=6) were 50% female and 50% male. The present study was approved by the Institutional Animal Care and Use Committee of The Second Hospital of Shandong University (Jinan, China).

Characterizing myocarditis by hematoxylin and eosin (H&E) staining. After obtaining mouse myocardial tissue samples (n=6), the myocardium tissues were fixed for 24 h using 10% formalin at room temperature and embedded in paraffin and sectioned to 3-5 μ m; tissue sections were floated in a water bath and placed onto glass slides. The glass slides were then placed in staining racks. Paraffin was cleared from the samples in three changes of xylene (2 min per change). After hydrating the samples, the sections were stained in hematoxylin solution for 1 min and were washed under running tap water at room temperature for \geq 5 min. Samples were then stained in working eosin Y solution for 10 sec, after which the samples were dehydrated and cleared in three changes of xylene (2 min per change). The slides were viewed under a light microscope. Myocarditis was characterized by inflammatory cell infiltration, myocardial cell degeneration, necrosis and a disordered myocardial arrangement (20).

Characterizing myocarditis by echocardiogram examination. Cardiac function and morphology were monitored by M- and B-mode transthoracic echocardiography, respectively (21). Left ventricular fractional shortening (LVFS) and left ventricular ejection fraction (LVEF) were assessed by cardiac magnetic resonance imaging in all mice (n=6/group).

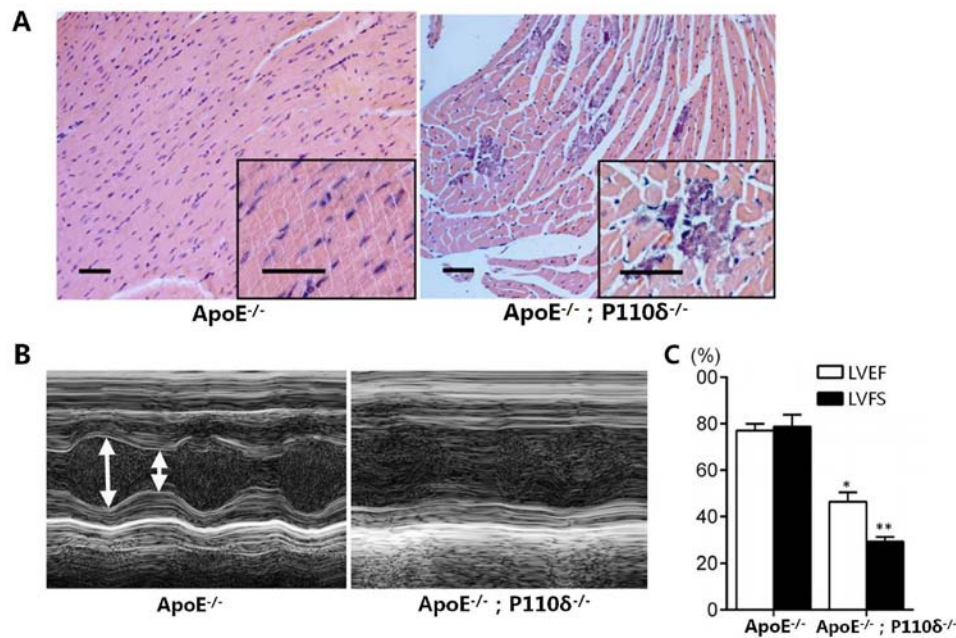


Figure 2. Deletion of P110 δ promotes myocarditis in mice. (A) Hematoxylin and eosin staining of myocardial cells in ApoE^{-/-} and ApoE^{-/-}/P110 δ ^{-/-} mice (n=6). Scale bar=1 μ l (B) Echocardiogram of the two groups of mice (n=6/group). (C) LVFS and LVEF of two groups of mice (n=6/group). *P<0.05 and **P<0.01 vs. ApoE^{-/-} mice. ApoE, apolipoprotein E; H&E, hematoxylin and eosin; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; P110 δ , phosphoinositide 3-kinase catalytic subunit δ isoform.

Counting of white blood cells and monocytes. Ascites were collected from the mice following sacrifice, and measurement of white blood cells was performed using an automated blood cell analyzer (Beckman Coulter, Inc., Brea, CA, USA). The whole blood was used to measure monocytes and neutrophils. The number of cells was expressed as means \pm standard deviation of three independent measurements.

Western blotting. Proteins were extracted from mouse myocardial tissues using general protein kits from Beyotime Institute of Biotechnology (Haimen, China). All protein samples were adjusted to equal concentrations via a Bicinchoninic Acid protein assay, followed by the addition of bromophenol blue. Equal amounts of proteins were loaded on 10% SDS-PAGE. A total of 6 μ l protein marker (EMD Millipore, Billerica, MA, USA) was added at the same time. The protein samples were separated according to a predetermined voltage. Subsequently, the protein was transferred to nitrocellulose membranes. Then the membrane was blocked in room temperature for 2 h with TBS containing 5% skim milk and 0.1% Tween-20, and incubated with primary antibodies against caspase-3 (ab13585), Bax (ab32503), Bcl-2 (ab32124) and GAPDH (ab8245) (Abcam, Cambridge, MA, USA) at a dilution of 1:1,000 at 4°C overnight, followed by incubation with horseradish-peroxidase-conjugated goat anti-rabbit (A11008, Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and anti-mouse IgG secondary antibodies (A-11077, Invitrogen; Thermo Fisher Scientific, Inc.) for 60 min at room temperature. Detection was performed using the LI-COR Odyssey Scanning Infrared Fluorescence Imaging system (LI-COR Biosciences, Lincoln, NE, USA).

Flow cytometry. The red blood cell lysis buffer, ACK Lysis Buffer (Beyotime Institute of Biotechnology) was added to the

blood samples, and centrifuged at 350 \times g for 5 min at 4°C. The precipitate was then washed three times with 0.5% bovine serum albumin (BSA, Beyotime Institute of Biotechnology) for 5 min. Anti-CD4-fluorescein isothiocyanate antibody (F1773, 1:100; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was added and incubated for 30 min at 4°C in the dark, followed by centrifugation at 350 \times g for 5 min at 4°C. The precipitate was washed with 0.5% BSA three times and the supernatant was discarded. The final precipitate was resuspended with 200 μ l 0.5% BSA for detection. The protein was detected using FACSCanto™ II (Becton Dickinson, Franklin Lakes, NJ, USA). The results are expressed as the means \pm standard deviation of three independent measurements.

Statistical analysis. All experimental data are expressed as the means \pm standard deviation and were analyzed by Image-Pro-Plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA) and Graph Pad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). One-way analysis of variance was used for multi-group comparisons followed by Bonferroni method as a post hoc test for multiple comparisons. Paired t-test was used to compare two groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Construction of a mouse model. Male ApoE^{-/-} mice and female P110 δ ^{-/-} mice (age, 6 weeks) were placed in one cage, and F1 mice were identified by DNA detection. Male and female ApoE^{-/-} mice from the F1 generation were hybridized with F1 generation P110 δ ^{-/-} mice to obtain the F2 generation. F2 generation mice were also identified with DNA detection. ApoE^{-/-} mice were selected as the control group, whereas ApoE^{-/-}/P110 δ ^{-/-} mice from the F2 generation were selected as

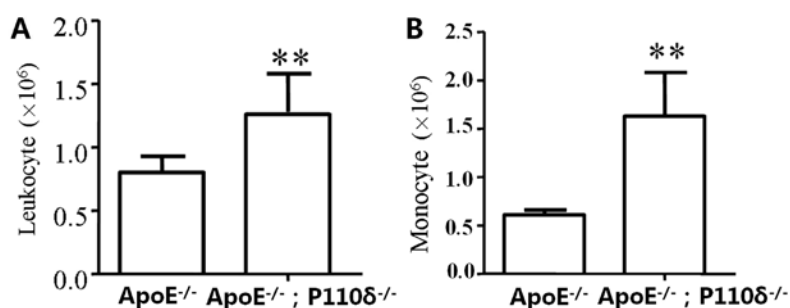


Figure 3. Deletion of P110 δ promotes inflammatory cell infiltration in mice in ApoE^{-/-} mice. (A) Blood cell analysis was used to detect alterations in leukocyte content in ApoE^{-/-} and ApoE^{-/-}/P110 δ ^{-/-} mice (n=6). (B) Cell analysis was used to detect alterations in monocyte content in ApoE^{-/-} and ApoE^{-/-}/P110 δ ^{-/-} mice (n=6). **P<0.01. ApoE, apolipoprotein E; P110 δ , phosphoinositide 3-kinase catalytic subunit δ isoform.

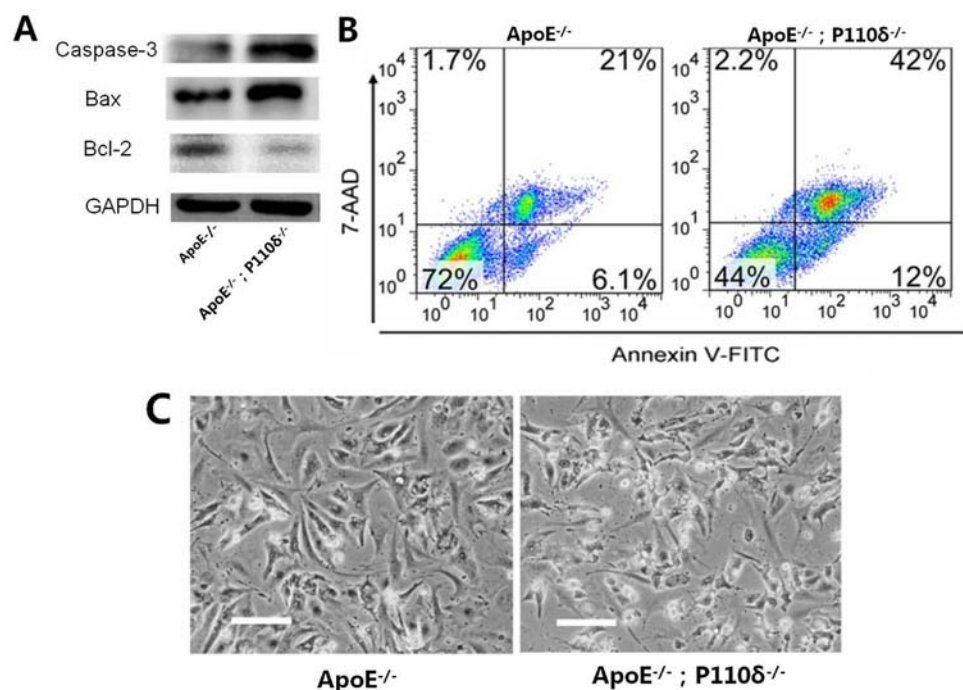


Figure 4. Deletion of P110 δ promotes apoptosis of mouse cardiomyocytes. (A) Apoptotic protein expression in ApoE^{-/-}/P110 δ ^{-/-} mice was detected by western blotting. (B) Percentage of apoptosis was detected by flow cytometry. (C) Morphology of mouse cardiomyocytes. Scale bar=5 μ m. 7-AAD, 7-amino-actinomycin D; ApoE, apolipoprotein E; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; FITC, fluorescein isothiocyanate; P110 δ , phosphoinositide 3-kinase catalytic subunit δ isoform.

the experimental group. The results of the identification are presented in Fig. 1A; lanes 1, 6, 7, 8, 9 and 10 are ApoE^{+/+} with two bands at 165 and 330 bp; lanes 2, 3, 4, 5, 11 and 12 are ApoE^{-/-} with one band at 330 bp. As shown in Fig. 1B, lanes 1, 2 and 3 are P110 δ ^{+/+} heterozygous with two bands at 145 and 485 bp; lanes 4, 5, 6, 7 and 8 are P110 δ ^{-/-} homozygous with one band at 485 bp.

Deletion of P110 δ promotes myocarditis in mice. P110 δ is involved in T and B cell differentiation, lymphocyte maturation and the neutrophil chemotaxis process. Therefore, the present study aimed to investigate whether the absence of P110 δ would induce myocarditis in ApoE^{-/-} mice. As shown in Fig. 2A, cells were spindle-shaped and regular in ApoE^{-/-} mice; however, in the ApoE^{-/-}/P110 δ ^{-/-} mice the nuclei of cells were smaller and the number of inflammatory cells was markedly increased. The electrocardiogram results presented in Fig. 2B

demonstrated that contractions in the control group were normal, whereas in the ApoE^{-/-}/P110 δ ^{-/-} mice, the systolic and diastolic contractions were notably smaller; and further examination indicated that LVFS and LVEF were decreased by 38.5 and 61.3% compared with in the control group (Fig. 2C).

Deletion of P110 δ promotes infiltration of inflammatory cells. H&E staining revealed that infiltration of inflammatory cells occurs in P110 δ -deficient mice. Leukocyte adhesion and infiltration is achieved through endothelial cells; therefore, these functional alterations may lead to a series of heart diseases. In particular, monocytes serve an important role in initiating cell adhesion. Therefore, peritoneal cells were extracted and analyzed in the present study. Blood cell analysis demonstrated that the amount of white blood cells in the experimental group was significantly higher than in the control group, and there was a statistically significant difference (Fig. 3A). In addition,

the number of monocytes in ApoE^{-/-}/P110δ^{-/-} mice was significantly increased compared with in the control group (Fig. 3B).

Deletion of P110δ promotes apoptosis of mouse cardiomyocytes. Apoptosis is a process of programmed cell death, which induced by naturally occurring cellular processes that is different from pathological cell necrosis. Apoptosis of myocardial cells in the two mouse groups was analyzed. Western blotting demonstrated that the expression levels of Bcl-2 in ApoE^{-/-}/P110δ^{-/-} mice were markedly decreased compared with in the control group, while the expression levels of Bax and caspase-3 were notably increased within ApoE^{-/-}/P110δ^{-/-} mice (Fig. 4A). As shown in Fig. 4B, apoptosis of myocardial cells in ApoE^{-/-}/P110δ^{-/-} mice was increased by 2-fold compared with in the control group as measured by flow cytometry. In addition, the morphology of myocardial cells in the control group were spindle-shaped, whereas cells in the experimental group appeared shrunken and apoptotic by florescence microscopy (Fig. 4C). Therefore, it may be hypothesized that deletion of P110δ promotes apoptosis of mouse cardiomyocytes in ApoE^{-/-}/P110δ^{-/-} mice.

Discussion

Numerous clinical and experimental animal studies have confirmed that inflammation serves an important role in the pathophysiology of myocarditis (22,23). In an experimental model of myocarditis, it was revealed that inflammatory cells, such as monocytes, macrophages and dendritic cells, may infiltrate the vascular adventitia. These inflammatory cells could improve myocarditis by increasing the infiltration of inflammatory cells into the adventitia (24). The present study indicated that cardiomyocytes were significantly infiltrated by inflammatory cells and the nuclei were clearly disorganized in ApoE^{-/-}/P110δ^{-/-} mice (data not shown). An electrocardiogram revealed that the control group exhibited normal systolic contractions; however, in ApoE^{-/-}/P110δ^{-/-} mice systole and diastole were markedly reduced, and LVFS and LVEF were significantly decreased. Furthermore, the results revealed that ApoE^{-/-}/P110δ^{-/-} mice had a significant increase in leukocyte and monocyte levels. Apoptosis is a process of programmed cell death. A previous study reported that anti-apoptotic drugs could pass the blood-brain barrier to reach the drug-target site, thereby reducing intracranial excitotoxicity and release of cytochrome *c*. Blocking the release of cytochrome *c* would prevent the transcriptional translation of apoptotic genes, thereby increasing the expression of the anti-apoptotic protein, Bcl-2, through a series of cascade reactions, which have a role in the regulation of apoptosis (25). Western blotting demonstrated that the levels of apoptotic proteins, such as Bax and caspase-3, were significantly increased in ApoE^{-/-}/P110δ^{-/-} mice compared with in the control group. Apoptosis of myocardial cells in ApoE^{-/-}/P110δ^{-/-} mice was also higher than that in the control group (42 vs. 21%). Furthermore, the morphology of myocardial cells was spindle-shaped in the control group, whereas, the cells were shrunken and apoptotic in the experimental group. Therefore, it may be hypothesized that deletion of P110δ promotes apoptosis of mouse cardiomyocytes in ApoE^{-/-}/P110δ^{-/-} mice.

In conclusion, it may be hypothesized that deletion of P110δ is an inducing factor in the development of myocarditis in mice. In addition, P110δ may have an important role in the absence of ApoE. The mechanism of action underlying the development of myocarditis may be associated with monocyte infiltration and apoptosis.

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Availability of data and materials

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

Authors' contributions

QZZ, AYY, WW and FL conducted the experiments. QZZ, AYY, AMP, LNC and WW drafted the manuscript and acquired and analyzed the data, and made substantial contributions to the concept and design of the present study. QZZ, AYY, WW, AMP, LNC and FL interpreted the data and revised the manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Animal Care and Use Committee of The Second Hospital of Shandong University (Jinan, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Mensah K and Anderson JR: Diagnostic investigations of adult cardiac disease. *Surg (Oxford)* 36: 52-56, 2018.
2. Chen QQ, Chen M, Zhang LH, Zeng Y, Qi-Cai L, Yang LQ and Lin XC: Costimulation blockade by combining CTLA4Ig with anti-CD40L mAb markedly inhibits the inflammatory response of experimental autoimmune myocarditis. *Eur J Inflamm* 15: 28-34, 2017.
3. Ramos C, Becerril C, Montañó M, García-De-Alba C, Ramírez R, Checa M, Pardo A and Selman M: FGF-1 reverts epithelial-mesenchymal transition induced by TGF-β1 through MAPK/ERK kinase pathway. *Am J Physiol Lung Cell Mol Physiol* 299: L222-L231, 2010.
4. Adamson IY, Bowden DH, Cote MG and Witschi H: Lung injury induced by butylated hydroxytoluene: cytodynamic and biochemical studies in mice. *Lab Invest* 36: 26-32, 1977.
5. Bachmeier C, Paris D, Beaulieu-Abdelahad D, Mouzon B, Mullan M and Crawford F: A multifaceted role for apoE in the clearance of beta-amyloid across the blood-brain barrier. *Neurodegener Dis* 11: 13-21, 2013.

6. Bangen KJ, Beiser A, Delano-Wood L, Nation DA, Lamar M, Libon DJ, Bondi MW, Seshadri S, Wolf PA and Au R: APOE genotype modifies the relationship between midlife vascular risk factors and later cognitive decline. *J Stroke Cerebrovasc Dis* 22: 1361-1369, 2013.
7. Bell RD, Winkler EA, Singh I, Sagare AP, Deane R, Wu Z, Holtzman DM, Betsholtz C, Armulik A, Sallstrom J, *et al*: Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature* 485: 512-516, 2012.
8. Bender AR and Raz N: Age-related differences in memory and executive functions in healthy APOE ϵ 4 carriers: The contribution of individual differences in prefrontal volumes and systolic blood pressure. *Neuropsychologia* 50: 704-714, 2012.
9. Vanhaesebroeck B, Leeyers SJ, Ahmadi K, Timms J, Katso R, Driscoll PC, Woscholski R, Parker PJ and Waterfield MD: Synthesis and function of 3-phosphorylated inositol lipids. *Annu Rev Biochem* 70: 535-602, 2001.
10. Marone R, Cmiljanovic V, Giese B and Wymann MP: Targeting phosphoinositide 3-kinase: Moving towards therapy. *Biochim Biophys Acta* 1784: 159-185, 2008.
11. Schu PV, Takegawa K, Fry MJ, Stack JH, Waterfield MD and Emr SD: Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting. *Science* 260: 88-91, 1993.
12. Kok K, Nock GE, Verrall EA, Mitchell MP, Hommes DW, Peppelenbosch MP and Vanhaesebroeck B: Regulation of p110delta PI 3-kinase gene expression. *PLoS One* 4: e5145, 2009.
13. Vanhaesebroeck B, Welham MJ, Kotani K, Stein R, Warne PH, Zvelebil MJ, Higashi K, Volinia S, Downward J and Waterfield MD: P110delta, a novel phosphoinositide 3-kinase in leukocytes. *Proc Natl Acad Sci USA* 94: 4330-4335, 1997.
14. Harris SJ, Foster JG and Ward SG: PI3K isoforms as drug targets in inflammatory diseases: Lessons from pharmacological and genetic strategies. *Curr Opin Investig Drugs* 10: 1151-1162, 2009.
15. Al-Rasheed NM, Fadda LM, Attia HA, Ali HM and Al-Rasheed NM: Quercetin inhibits sodium nitrite-induced inflammation and apoptosis in different rats organs by suppressing Bax, HIF1- α , TGF- β , Smad-2, and AKT pathways. *J Biochem Mol Toxicol* 31, 2017.
16. Berndt A, Miller S, Williams O, Le DD, Houseman BT, Pacold JJ, Gorrec F, Hon WC, Liu Y, Rommel C, *et al*: The p110 delta structure: Mechanisms for selectivity and potency of new PI(3)K inhibitors. *Nat Chem Biol* 6: 117-124, 2010.
17. Kang HR, Cho SJ, Lee CG, Homer RJ and Elias JA: Transforming growth factor (TGF)- β 1 stimulates pulmonary fibrosis and inflammation via a Bax-dependent, bid-activated pathway that involves matrix metalloproteinase-12. *J Biol Chem* 282: 7723-7732, 2007.
18. Tang F, Li X, Gui Y, Qi C, Lu M, Dai C, Wang H and Wang L: p110Delta inhibits monocyte infiltration by thioglycollate-induced peritoneal inflammation but not HCD-induced inflammation and atherosclerosis in APOE KO mice. *Lipids* 50: 839-846, 2015.
19. Bucher K, Schmitt F, Mothes B, Blumendeller C, Schäll D, Piekorz R, Hirsch E, Nürnberg B and Beer-Hammer S: Deficiency of PI3-Kinase catalytic isoforms p110 γ and p110 δ in mice enhances the IL-17/G-CSF axis and induces neutrophilia. *Cell Commun Signal* 15: 28, 2017.
20. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S and Schmid B.: Fiji: An open-source platform for biological-image analysis. *Nat Methods* 9: 676-682, 2012.
21. Sehgal A, Allison BJ, Gwini SM, Miller SL and Polglase GR: Cardiac morphology and function in preterm growth restricted infants: Relevance for clinical sequelae. *J Pediatr* 188: 128-134, 2017.
22. Hueso M, De Ramon L, Navarro E, Ripoll E, Cruzado JM, Grinyo JM and Torras J: Datasets for the validation of the 'in vivo' siRNA-silencing of CD40 and for the detection of new markers of atherosclerosis progression in ApoE-deficient mice. *Data Brief* 9: 1105-1112, 2016.
23. Li M, Su Y, Yu Y, Wang X, Zou Y, Ge J and Chen R: Dual roles of calpain in facilitating Coxsackievirus B3 replication and prompting inflammation in acute myocarditis. *Int J Cardiol* 221: 1123-1131, 2016.
24. Heymans S, Eriksson U, Lehtonen J and Cooper LT Jr: The quest for new approaches in myocarditis and inflammatory cardiomyopathy. *J Am Coll Cardiol* 68: 2348-2364, 2016.
25. Wang Y, Liu L, Yang L and Wang H: Dracorhodin perchlorate induced human breast cancer MCF-7 cell apoptosis through mitochondrial pathway. *Mod Chin Med* 17: 1263-1266, 2015.



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