Analysis of Signaling Pathways of Necroptotic and Pyroptotic Cell Death in the Hearts of Rats With Type 2 Diabetes Mellitus

Csaba HORVÁTH[1], Izabela JARABICOVÁ[1], Tomáš RAJTÍK[1], Linda BARTOŠOVÁ[1], Kristína FERENCZYOVÁ[2], Barbora KALOČAYOVÁ[2], Monika BARTEKOVÁ[2,3], Adrián SZOBI[1], Adriana ADAMEOVÁ[1,2]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University in Bratislava, Bratislava, Slovak Republic, [2]Centre of Experimental Medicine, Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic, [3]Institute of Physiology, Faculty of Medicine, Comenius University in Bratislava, Bratislava, Slovak Republic

Received September 7, 2022
Accepted November 7, 2022

Summary

Diabetes mellitus is known to produce various cell-damaging events and thereby underlie heart dysfunction and remodeling. However, very little is known about its inflammation-associated pathomechanisms due to necrosis-like cell death. For this purpose, we aimed to investigate signaling pathways of necroptosis and pyroptosis, known to produce plasma membrane rupture with the resultant promotion of inflammation. One-year old Zucker diabetic fatty (ZDF) rats did not exhibit significant heart dysfunction as revealed by echocardiographic measurement. On the other hand, there was a decrease in heart rate due to diabetes. Immunoblotting analysis showed that the left ventricles of ZDF rats overexpress neither the main necroptotic proteins including receptor-interacting protein kinase 3 (RIP3) and mixed lineage domain kinase-like pseudokinase (MLKL), nor the pyroptotic regulators including NLR family pyrin domain containing 3 protein (NLRP3), caspase-1, interleukin-1 beta (IL-1β and the N-terminal gasdermin D (GSDMD-N). On the other hand, the increased activation of the RIP3 kinase due to phosphorylation was found in such hearts. In summary, we showed for the first time that the activation of cardiac RIP3 is upregulated due to disturbances in glucose metabolism which, however, did not proceed to necrosis-like cell death. These data can indicate that the activated RIP3 might also underlie other pleiotropic, non-necroptotic signaling pathways under basal conditions.

Key words

Necroptosis • Pyroptosis • Diabetes mellitus • Heart

Corresponding author

A. Adameová, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University in Bratislava, 81499 Bratislava, Slovak Republic. E-mail: adriana.duris.adameova@uniba.sk

Introduction

Cell death has been shown to be a critical and significant contributor to the pathomechanisms of various cardiovascular diseases [1-5]. In recent years, a conservative view on cell death classification involving chaotic necrotic and regulated apoptotic cell loss has advanced. Indeed, other necrosis-like cell death forms – necroptosis and pyroptosis, which are strictly regulated but resemble necrotic phenotypes, have been introduced. The process of necroptosis follows the auto- or receptor-interacting protein kinase-1 (RIP1)-mediated phosphorylation of RIP3 which subsequently phosphorylates, thus activates the terminal necroptotic executioner mixed lineage domain kinase-like pseudokinase (MLKL) [6,7]. Such activated MLKL forms homo-oligomers or hetero-oligomers with RIP3 which accumulate within the plasma membrane resulting in the loss of its integrity and finally cell lysis [6,8]. The induction of pyroptosis is triggered by the formation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome causing caspase-1-mediated maturation of pro-inflammatory cytokines, such as interleukin-1 beta

(IL-1β). Furthermore, activated caspase-1 cleaves gasdermin D to generate an N-terminal cleavage product (GSDMD-N) that induces the plasma membrane rupture and release of inflammatory cytokines, thereby highlighting the pro-inflammatory character of pyroptotic cell death [9]. Although there are some not very well-defined mechanisms leading to the activation of these cell death forms, it is clear that both these regulated necrotic processes proceed to cell loss by promoting the release of the intracellular content and underlying pro-inflammatory environment [3,10-13].

Several conventional mechanisms, such as altered Ca^{2+} homeostasis [14,15], oxidative stress [16,17], altered function of proteolytic proteins [18] have been suggested to be implemented in the pathomechanisms of cardiac damage due to diabetes mellitus (DM). Recently, inflammation has also been suggested to contribute, at least in part, to changes mediated by DM [19]. Thus, in view of these observations, we aimed to describe the main pronecroptotic and pyroptotic signaling pathways, as potential sources of inflammation, in the heart of rats experiencing type 2 DM. In this regard it can be mentioned that deleterious effects of necroptosis and pyroptosis have been shown to underlie the altered function and adverse remodeling due to ischemia/reperfusion and pulmonary arterial hypertension under normoglycemic conditions [12,20,21]. These findings might bring more insights on DM-mediated influence on the heart function and indicate perspectives on future therapies for this disease.

Methods

Experimental model

One-year-old obese Zucker diabetic fatty (ZDF) rats (fa/fa) (Diabetes group, n=7) and their age-matched non-diabetic lean controls (fa/+) (Control group, n=6) obtained from Dobrá Voda, Slovak Republic, were used in the study. After the adaptation period at stable conditions (temperature of 22±2 °C and humidity of 45-65 %, water *ad libitum*), rats were anaesthetized with thiopental (50 mg/kg, i.p.) in combination with heparin (500 IU, s.c.) and their hearts were quickly excised and stored at -80 °C until further analyses. Biometric data including bodyweight, fasting glycemia and cholesterol levels were collected and calculated before euthanasia. All experiments were performed in accordance with the rules issued by the State Veterinary Administration of the Slovak Republic, legislation No. 377/2012 and with the regulations of the Animal Research and Care Committee of Centre of Experimental Medicine SAS – Project No. 2237/18-221/3, approved on 21 August 2018.

Echocardiography

Echocardiographic examination was performed on anesthetized rats put in a chamber with a continuous supply of isoflurane (Forane, Abbive, USA). The data were obtained using the GE Healthcare Vivid E9 ultrasound machine (GE Healthcare, USA) with a 15.0-MHz transducer probe. During the echocardiography exam, images of parasternal long axis (PLAX), short axis (SAX) and apical four chambers (A4C) were captured using two-dimensional mode (2-D), M-mode, Color Doppler and Pulse wave Doppler. All exams were performed during short and controlled period to ensure highly accurate data. Captured images were subsequently analyzed by EchoPac software (GE Healthcare, USA) obtaining the data depicting heart rate as well as ejection fraction of the rats.

Immunoblotting

Immunoblot analysis was performed on whole cell lysates from the left ventricles as described previously [20]. Briefly, following post-electrophoretic transfer of proteins, the membranes were incubated with the following primary antibodies against caspase-1 (ab179515, Abcam, UK), GSDMD (sc-81868, Santa Cruz, USA), IL-1β (ab9722, Abcam, UK), MLKL (ab243142, Abcam, UK), NLRP3 (ab263899, Abcam, UK), pSer345-MLKL (MABC-1158, Merck, Germany), pThr231/Ser232-RIP3 (ab222320, Abcam, UK) and RIP3 (#15828, Cell Signaling Technology, USA). Subsequently, the membranes were incubated with the following secondary HRP-conjugated antibodies: donkey anti-rabbit IgG (711-035-152, Jackson Immunoresearch), donkey anti-rat IgG (112-035-175, Jackson Immunoresearch, USA) and donkey anti-mouse IgG (115-035-174, Jackson Immunoresearch, USA). Signals were detected using enhanced chemiluminescence (Crescendo Luminata, Merck Millipore, USA) and captured by a chemiluminescence imaging system (myECL imager, Thermo Scientific, USA). Total protein staining of membranes with Ponceau S assessed by scanning densitometry was used as the loading control in total tissue lysates [22]. Relative expression of protein bands of interest was calculated by normalizing the intensity of a protein band with its whole lane protein staining intensity.

Statistical analysis

Data are expressed as means ± standard error of means (SEM) for the number of animals in the group. Unpaired *t*-test with Welch's correction was used to compare the differences in variables with normal distribution between the 2 groups using GraphPad Prism 9.00 for Windows (GraphPad Software, USA). Differences between the groups were considered significant when $p<0.05$.

Results

Basic characteristics

Biometric data are shown in Table 1. As expected, fasting glycemia (5.93±0.18 mmol/l vs. 13.22±2.19 mmol/l) and cholesterol levels (2.87±0.18 mmol/l vs. 5.12±0.26 mmol/l) were significantly higher in ZDF rats in comparison to the control group. Likewise, these diabetic rats exhibited significantly increased body weight (411.33±18.53 g vs. 576.33±39.39 g). Collectively, 1-year-old ZDF rats exhibited obesity and disturbed metabolism of glucose and cholesterol.

Echocardiographic data

Main echocardiographic parameters including a representative image are depicted in Figure 1A-D. Ejection fraction (EF) and relative wall thickness (RWT) were comparable between the groups indicating no significant heart dysfunction and remodeling under these particular conditions in this stage of the disease (Fig. 1A, B). Other echocardiographic parameters have also not advocated for the alterations in cardiac function either in the size of left ventricle (data not shown). On the other hand, decreased heart rate in obese ZDF rats indicated the presence of bradycardia (Fig. 1C).

Necroptotic and pyroptotic signaling

Figures 2A-C and 3A-D show the detailed analysis of the canonical pathway of necroptosis and pyroptosis, respectively. The levels of total RIP3 were

Table 1. Biometric data of rats. Data are expressed as mean ± SEM (n=6-7/group); * p<0.05; ** p<0.01.

	Control	Diabetes
Bodyweight [g]	411.33 ± 18.53	576.33 ± 39.39**
Fasting glycemia [mmol/l]	5.93 ± 0.18	13.22 ± 2.19*
Cholesterol [mmol/l]	2.87 ± 0.18	5.12 ± 0.26**

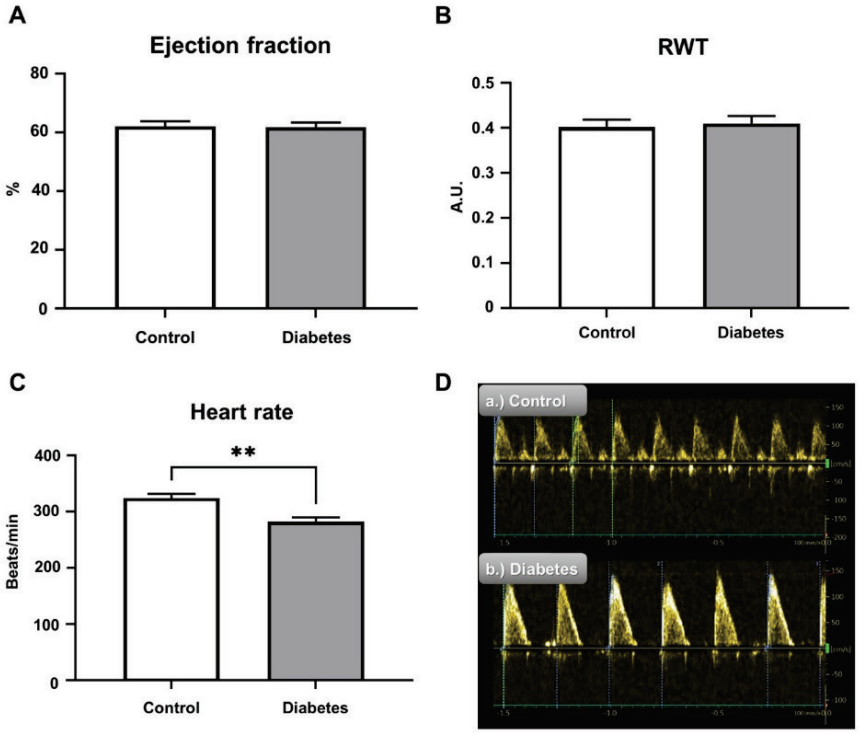

Fig. 1. Echocardiographic parameters of rats with type 2 diabetes. (**A**) Ejection fraction expressed as percentage; (**B**) Relative wall thickness (RWT) expressed as A.U.; (**C**) Heart rate expressed as beats/min; (**D**) Representative echocardiographic image. Data are expressed as mean ± SEM (n=6-7/group); * p<0.05; ** p<0.001.

unchanged, but its phosphorylated form, which is known to promote necroptosis, showed an increasing trend in the diabetic hearts in comparison to the control group. In support, the ratio of pThr231/Ser232-RIP3 to total RIP3, a strong indicator towards the activation of this multifaceted kinase [23], was significantly upregulated in the LVs of diabetic rats (Fig. 2A). However, such activation unlikely proceeded to necroptosis execution since the phosphorylated form of MLKL was unchanged in both groups (Fig. 2B).

The expression of neither procaspase-1 nor its cleaved active form caspase-1 was changed in the hearts due to DM (Fig. 3A). Similar results were observed in both the inactive and active form of its main cleavage target – IL-1β (Fig. 3B). In line, neither the expression of the pyroptotic initiator NLRP3, nor its final executioner protein the N-terminal GSDMD, producing the plasma membrane rupture, showed changes between the groups (Fig. 3C).

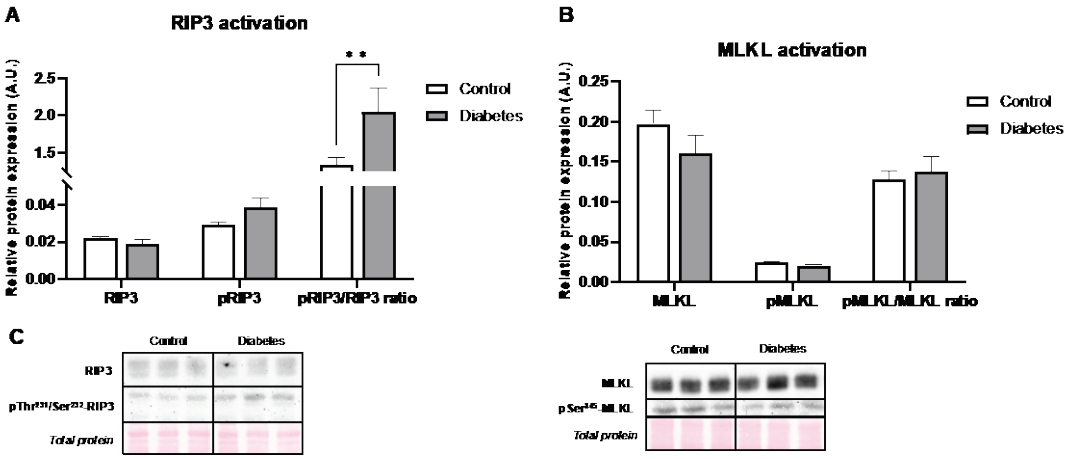

Fig. 2. Analysis of necroptosis activation in the left ventricles of rats with type 2 diabetes. (**A**) Immunoblot analysis of the expression of total RIP3, pThr231/Ser232-RIP3 and pThr231/Ser232-RIP3/RIP3 ratio; (**B**) Immunoblot analysis of the expression of total MLKL, pSer345-MLKL and pSer345-MLKL/MLKL ratio; (**C**) Representative immunoblots. Data are expressed as mean ± SEM (n=6-7/group); ** p<0.001.

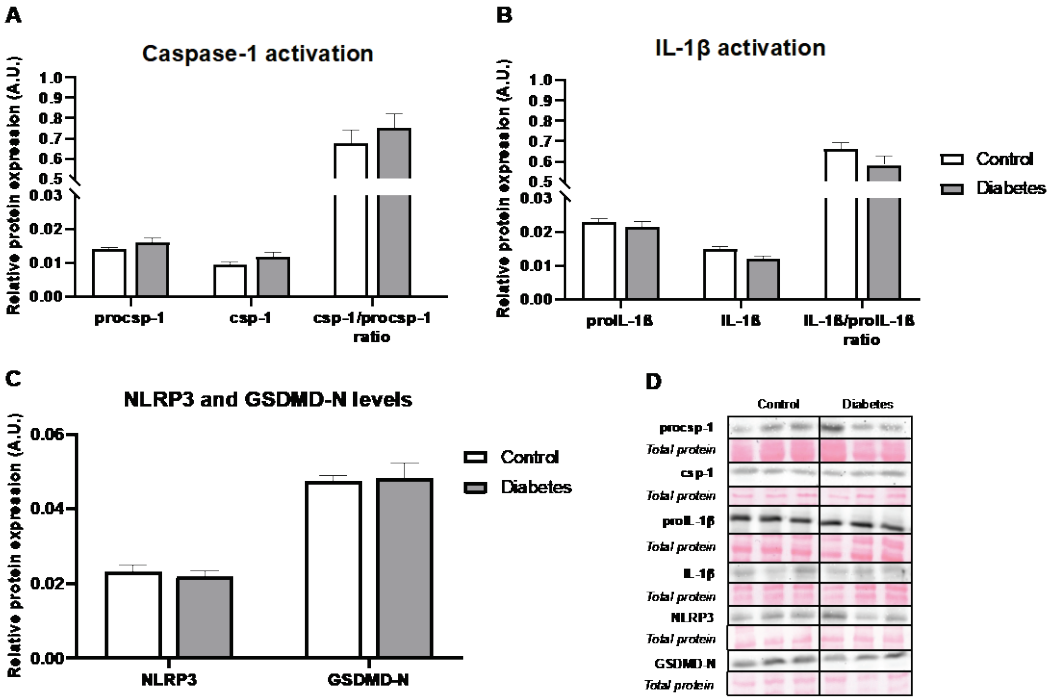

Fig. 3. Analysis of pyroptosis activation in the left ventricles of rats with type 2 diabetes. (**A**) Immunoblot analysis of the expression of procsp-1, csp-1, csp-1/procsp1 ratio, pro IL-1β, IL-1β and IL-1β/proIL-1β ratio; (**B**) Immunoblot analysis of the expression of NLRP3 and GSDMD-N; (**C**) Representative immunoblots. Data are expressed as mean ± SEM (n=6-7/group).

Together, these molecular analyses in 1-year-old obese ZDF rats with disturbed glucose and cholesterol metabolism, have indicated the increased activation of RIP3 by phosphorylation however, necroptosis or pyroptosis are unlikely executed under such conditions.

Discussion

The present study was carried out to extend the knowledge about myocardial inflammation-associated cell death signaling in type 2 diabetes and thereby assess the likely predisposition to cell loss and subsequent cardiac damage. Complex analysis of necroptotic and pyroptotic cell death modes revealed for the first time that the LVs of obese ZDF rats do not overexpress the main proteins of these signaling pathways under basal conditions. Notably, the higher activation of the RIP3 kinase due to phosphorylation was observed in the hearts, only. Thus, these data might indicate that such activated RIP3 in the heart of obese ZDF rats could be involved in other cell signaling pathways, such as mitochondrial swelling [20], and that further pathological stimuli (e.g. ischemia) are needed to promote RIP3-mediated necrosis-like cell death and thereby underlie heart dysfunction.

The relevance of necroptotic loss of cells has been documented in some studies employing models of prediabetes [24] and diabetes [25]. In a 12-week high fat diet-induced pre-diabetic rat model, inhibition of RIP1, an upstream activator of RIP3, reversed the adverse effects of the disease in terms of improving cognitive function, synaptic plasticity, and mitochondrial function in the brain [24]. Likewise, islets treated with necroptosis inhibitor produced and secreted more insulin which was accompanied by the upregulation of GLUT2 expression in comparison with non-treated islets [26]. In support, inhibition of RIP1 and gene knockout of MLKL enhanced hepatic insulin sensitivity and ameliorated insulin resistance [25]. These studies have indicated an important role of necroptosis in cell death in the brain, liver, and islets in pre-diabetes/diabetes. Regarding the heart, cardiomyocytes isolated from RIP3 or MLKL deficient mice with type 1 diabetes were protected from excessive necroptotic loss [27]. Interestingly, the activity of RIP1 and RIP3 was also associated with increased myocardial fibrosis due to disturbed autophagic flux in streptozotocin-induced diabetes of Sprague-Dawley rats [28]. In this study, by analyzing basal necroptotic signaling we intended to assess the sensitivity of the heart to cell loss due to the metabolic complications only, and thus, with no additional pathologic stimuli. Because there are no changes found in the expression of either of the proteins of necroptotic signaling, including phosphorylated MLKL, it is very likely that such disturbances in glucose and cholesterol metabolism do not produce deleterious effects in terms of the basal loss of cardiac cells. In this regard it can be mentioned that neither diet-induced hypercholesterolemia was able to increase the expression of main necroptotic proteins in the heart under basal conditions [29]. In addition, here we did not find the higher levels of markers of pyroptosis in the hearts of obese ZDF rats either. Although such finding might seem contrary with other studies showing that the NLRP3 inflammasome is activated in diabetic cardiomyopathy indicating a relevance for pyroptotic cell loss [30-32], these studies investigated a streptozotocin-induced model of diabetes while our study used a model for type 2 diabetes.

Because we observed the higher phosphorylation of RIP3 due to the plasma alterations in glucose levels, and because it did not produce necrosis-like cell death under basal conditions, it can be hypothesized that such activated RIP3 can be involved in other cell damaging mechanisms. In addition, it can be hypothesized that there is an association between the upregulation of the phosphorylated levels of RIP3 and oxidative stress. In fact, a link between reactive oxygen species (ROS), which are elevated in DM [16] and the activation of RIP3 has been nicely documented elsewhere [33,34]. Considering this particular role of phosphorylated form of RIP3 in mitochondrial swelling and ROS pathogenesis, it can be concluded that non-cell death associated events of the activated RIP3 are likely mechanisms underlying alterations in signaling pathways due to DM under basal conditions. Likewise, it can be postulated that additional pathological stimuli (e.g. ischemia) are needed to promote RIP3-mediated necrosis-like cell death and thereby underlie heart dysfunction and remodeling progressing in diabetic cardiomyopathy. Further investigation is needed to prove this concept of a pleiotropic action of RIP3 in type 2 diabetes.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This research was funded by grants APVV-15-0607, APVV-20-0242, APVV-19-0540, APVV-21-0194, VEGA 1/0016/20, 2/0104/20 and 2/0104/22.

References

1. Adameova A, Hrdlicka J, Szobi A, Farkasova V, Kopaskova K, Murarikova M, Neckar J, Kolar F, Ravingerova T, Dhalla NS. Evidence of necroptosis in hearts subjected to various forms of ischemic insults. Can J Physiol Pharmacol 2017;95:1163-1169. https://doi.org/10.1139/cjpp-2016-0609
2. Chai R, Xue W, Shi S, Zhou Y, Du Y, Li Y, Song Q, Wu H, Hu Y. Cardiac remodeling in heart failure: Role of pyroptosis and its therapeutic implications. Front Cardiovasc Med 2022;9:870924. https://doi.org/10.3389/fcvm.2022.870924
3. Patel P, Karch J. Regulation of cell death in the cardiovascular system. Int Rev Cell Mol Biol 2020;353:153-209. https://doi.org/10.1016/bs.ircmb.2019.11.005
4. Morgan MJ, Liu Z-G. Programmed cell death with a necrotic-like phenotype. Biomol Concepts 2013;4:259-275. https://doi.org/10.1515/bmc-2012-0056
5. Zhe-Wei S, Li-Sha G, Yue-Chun L. The role of necroptosis in cardiovascular disease. Front Pharmacol 2018;9:721. https://doi.org/10.3389/fphar.2018.00721
6. Wang H, Sun L, Su L, Rizo J, Liu L, Wang LF, Wang FS, Wang X. Mixed lineage kinase domain-like protein MLKL causes necrotic membrane disruption upon phosphorylation by RIP3. Mol Cell 2014;54:133-146. https://doi.org/10.1016/j.molcel.2014.03.003
7. Wu XN, Yang ZH, Wang XK, Zhang Y, Wan H, Song Y, Chen X, Shao J, Han J. Distinct roles of RIP1-RIP3 hetero- and RIP3-RIP3 homo-interaction in mediating necroptosis. Cell Death Differ 2014;21:1709-1720. https://doi.org/10.1038/cdd.2014.77
8. Dondelinger Y, Declercq W, Montessuit S, Roelandt R, Goncalves A, Bruggeman I, Hulpiau P, ET AL. MLKL compromises plasma membrane integrity by binding to phosphatidylinositol phosphates. Cell Rep 2014;7:971-981. https://doi.org/10.1016/j.celrep.2014.04.026
9. Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H, Lieberman J. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. Nature 2016;535:153-158. https://doi.org/10.1038/nature18629
10. Wang Q, Wu J, Zeng Y, Chen K, Wang C, Yang S, Sun N, Chen H, Duan K, Zeng G. Pyroptosis: A pro-inflammatory type of cell death in cardiovascular disease. Clin Chim Acta 2020;510:62-72. https://doi.org/10.1016/j.cca.2020.06.044
11. Del Re DP, Amgalan D, Linkermann A, Liu Q, Kitsis RN. Fundamental mechanisms of regulated cell death and implications for heart disease. Physiol Rev 2019;99:1765-1817. https://doi.org/10.1152/physrev.00022.2018
12. Lichý M, Szobi A, Hrdlička J, Horváth C, Kormanová V, Rajtík T, Neckář J, Kolář F, Adameová A. Different signalling in infarcted and non-infarcted areas of rat failing hearts: A role of necroptosis and inflammation. J Cell Mol Med 2019;23:6429-6441. https://doi.org/10.1111/jcmm.14536
13. Lawlor KE, Khan N, Mildenhall A, Gerlic M, Croker BA, D'Cruz AA, Hall C, ET AL. RIPK3 promotes cell death and NLRP3 inflammasome activation in the absence of MLKL. Nat Commun 2015;6:6282. https://doi.org/10.1038/ncomms7282
14. Belke DD, Dillmann WH. Altered cardiac calcium handling in diabetes. Curr Hypertens Rep 2004;6:424-429. https://doi.org/10.1007/s11906-004-0035-3
15. Ravingerová T, Stetka R, Pancza D, Ulicná O, Ziegelhöffer A, Styk J. Susceptibility to ischemia-induced arrhythmias and the effect of preconditioning in the diabetic rat heart. Physiol Res 2000;49:607-616.
16. Giacco F, Brownlee M, Schmidt AM. Oxidative stress and diabetic complications. Circ Res 2010;107:1058-1070. https://doi.org/10.1161/CIRCRESAHA.110.223545
17. Kancirová I, Jašová M, Muráriková M, Sumbalová Z, Uličná O, Ravingerová T, Waczulíková I, Ziegelhöffer A, Ferko M. Cardioprotection induced by remote ischemic preconditioning preserves the mitochondrial respiratory function in acute diabetic myocardium. Physiol Res 2016;65(Suppl 5):S611-S619. https://doi.org/10.33549/physiolres.933533
18. Ahmed N, Babaei-Jadidi R, Howell SK, Beisswenger PJ, Thornalley PJ. Degradation products of proteins damaged by glycation, oxidation and nitration in clinical type 1 diabetes, Diabetologia 2005;48:1590-1603. https://doi.org/10.1007/s00125-005-1810-7

19. Tsalamandris S, Antonopoulos AS, Oikonomou E, Papamikroulis GA, Vogiatzi G, Papaioannou S, Deftereos S, Tousoulis D. The role of inflammation in diabetes: current concepts and future perspectives. Eur Cardiol 2019;14:50-59. https://doi.org/10.15420/ecr.2018.33.1
20. Horvath C, Young M, Jarabicova I, Kindernay L, Ferenczyova K, Ravingerova T, Lewis M, Suleiman MS, Adameova A. Inhibition of cardiac RIP3 mitigates early reperfusion injury and calcium-induced mitochondrial swelling without altering necroptotic signalling. Int J Mol Sci 2021;22:7983. https://doi.org/10.3390/ijms22157983
21. Jarabicová I, Horváth C, Veľasová E, Bies Piváčková L, Vetešková J, Klimas J, Křenek P, Adameová A. Analysis of necroptosis and its association with pyroptosis in organ damage in experimental pulmonary arterial hypertension. J Cell Mol Med 2022;26:2633-2645. https://doi.org/10.1111/jcmm.17272
22. Moritz CP. Tubulin or not tubulin: Heading toward total protein staining as loading control in Western blots. Proteomics 2017;17:201600189. https://doi.org/10.1002/pmic.201600189
23. Liu Y, Liu T, Lei T, Zhang D, Du S, Girani L, Qi D, Lin C, Tong R, Wang Y. RIP1/RIP3-regulated necroptosis as a target for multifaceted disease therapy (Review). Int J Mol Med 2019;44:771-786. https://doi.org/10.3892/ijmm.2019.4244
24. Jinawong K, Apaijai N, Wongsuchai S, Pratchayasakul W, Chattipakorn N, Chattipakorn SC. Necrostatin-1 mitigates cognitive dysfunction in prediabetic rats with no alteration in insulin sensitivity. Diabetes 2020;69:1411-1423. https://doi.org/10.2337/db19-1128
25. Xu H, Du X, Liu G, Huang S, Du W, Zou S, Tang D, ET AL. The pseudokinase MLKL regulates hepatic insulin sensitivity independently of inflammation. Mol Metab 2019;23:14-23. https://doi.org/10.1016/j.molmet.2019.02.003
26. Lau H, Corrales N, Alexander M, Mohammadi MR, Li S, Smink AM, de Vos P, Lakey JRT. Necrostatin-1 supplementation enhances young porcine islet maturation and in vitro function. Xenotransplantation 2020;27:e12555. https://doi.org/10.1111/xen.12555
27. Cao T, Ni R, Ding W, Ji X, Li L, Liao G, Lu Y, Fan G-C, Zhang Z, Peng T. MLKL-mediated necroptosis is a target for cardiac protection in mouse models of type-1 diabetes. Cardiovasc Diabetol 2022;21:165. https://doi.org/10.1186/s12933-022-01602-9
28. Qiao S, Hong L, Zhu Y, Zha J, Wang A, Qiu J, Li W, Wang C, An J, Zhang H. RIPK1-RIPK3 mediates myocardial fibrosis in type 2 diabetes mellitus by impairing autophagic flux of cardiac fibroblasts. Cell Death Dis 2022;13:147. https://doi.org/10.1038/s41419-022-04587-1
29. Giricz Z, Koncsos G, Rajtík T, Varga ZV, Baranyai T, Csonka C, Szobi A, Adameová A, Gottlieb RA, Ferdinandy P. Hypercholesterolemia downregulates autophagy in the rat heart. Lipids Health Dis 2017;16:60. https://doi.org/10.1186/s12944-017-0455-0
30. Luo B, Li B, Wang W, Liu X, Xia Y, Zhang C, Zhang M, Zhang Y, An F. NLRP3 gene silencing ameliorates diabetic cardiomyopathy in a type 2 diabetes rat model, PLoS One 201419;9:e104771. https://doi.org/10.1371/journal.pone.0104771
31. Yang F, Qin Y, Wang Y, Meng S, Xian H, Che H, Lv J, ET AL. Metformin inhibits the NLRP3 inflammasome via AMPK/mTOR-dependent effects in diabetic cardiomyopathy. Int J Biol Sci 2019;15:1010-1019. https://doi.org/10.7150/ijbs.29680
32. Sun Y, Ding S. NLRP3 Inflammasome in diabetic cardiomyopathy and exercise intervention. Int J Mol Sci 2021;22:1322. https://doi.org/10.3390/ijms222413228
33. Zhang DW, Shao J, Lin J, Zhang N, Lu BJ, Lin SC, Dong MQ, Han J. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science 2009;325:332-336. https://doi.org/10.1126/science.1172308
34. Yang Z, Wang Y, Zhang Y, He X, Zhong CQ, Ni H, Chen X, Liang Y, Wu J, Zhao S, Zhou D, Han J. RIP3 targets pyruvate dehydrogenase complex to increase aerobic respiration in TNF-induced necroptosis. Nat Cell Biol 2018;20:186-197. https://doi.org/10.1038/s41556-017-0022-y