Distinct Types of Cell Death and the Implication in Diabetic Cardiomyopathy

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Diabetic cardiomyopathy (DCM) is a chronic complication of diabetes mellitus, characterized by abnormalities of myocardial structure and function. Researches on the models of type 1 and type 2 diabetes mellitus as well as the application of genetic engineering technology help in understanding the molecular mechanism of DCM. DCM has multiple hallmarks, including hyperglycemia, insulin resistance, increased free radical production, lipid peroxidation, mitochondrial dysfunction, endothelial dysfunction, and cell death. Essentially, cell death is considered to be the terminal pathway of cardiomyocytes during DCM. Morphologically, cell death can be classified into four different forms: apoptosis, autophagy, necrosis, and entosis. Apoptosis, as type I cell death, is the fastest form of cell death and mainly occurs depending on the caspase proteolytic cascade. Autophagy, as type II cell death, is a degradation process to remove damaged proteins, dysfunctional organelles and commences by the formation of autophagosome. Necrosis is type III cell death, which contains a great diversity of cell death processes, such as necroptosis and pyroptosis. Entosis is type IV cell death, displaying “cell-in-cell” cytological features and requires the engulfing cells to execute. There are also some other types of cell death such as ferroptosis, parthanatos, netotic cell death, lysosomal dependent cell death, alkaliptosis or oxeiptosis, which are possibly involved in DCM. Drugs or compounds targeting the signals involved in cell death have been used in clinics or experiments to treat DCM. This review briefly summarizes the mechanisms and implications of cell death in DCM, which is beneficial to improve the understanding of cell death in DCM and may propose novel and ideal strategies in future.

Keywords: diabetic cardiomyopathy, cell death, apoptosis, autophagy, necrosis, entosis

INTRODUCTION

Diabetic cardiomyopathy (DCM) is manifested as specific abnormalities of myocardial structure and function in diabetic patients without hypertension or coronary artery disease in clinic. DCM is regarded as one of the most common complications of diabetes to increase the risk of heart failure (Paolillo et al., 2019). Hyperglycemia, insulin resistance, fatty acids, oxidative stress, inflammation, myocardial fibrosis and hypertrophy, mitochondrial dysfunction, endoplasmic
reticular stress, and endothelial dysfunction are possible molecular foundations for DCM (Wang et al., 2014; Cao et al., 2015; Chen et al., 2018; Li et al., 2018a). Reactive oxygen species (ROS) produced by fatty-acid oxidation or nicotinamide adenine dinucleotide phosphate (NADPH) oxidase induce cell death or tissue damage (Parim et al., 2019). Essentially, cell death is considered to be the terminal pathway of cardiomyocytes during DCM. Experimental models of DCM show that metabolic dysfunction, cardiac structural, or functional abnormalities are similar to the pathology of human DCM. Streptozotocin (STZ) is the most common agent to induce type 1 diabetes mellitus (T1DM). Zucker diabetic fatty rat, leptin receptor deficiency mice (db/db), and leptin deficiency (ob/ob) mice are useful models to study type 2 DM (T2DM). Recently, echocardiography, computed tomography, magnetic resonance imaging, and single-photon emission computed tomography are used for the diagnosis of DCM (Adingupu et al., 2019; Parim et al., 2019; Tan et al., 2019). In vitro, DCM is often imitated in rat embryonic heart derived H9C2 cells, neonatal cardiomyocytes or cardiac fibroblasts with high glucose (HG) or advance glycation end product (AGE) stimulation (Sun et al., 2019a; Tang et al., 2019b; Wang et al., 2019a).

A large number of studies have shown several distinct types of cardiomyocyte death. Morphologically, cell death can be classified into four different forms. (1) Type I or apoptosis, featuring with cytoplasmic shrinkage, nuclear pyknosis, karyorrhexis, DNA fragmentation, and plasma membrane blebbing, and eventually apoptotic body formation. (2) Type II, or autophagy, showing extensive cytoplasmic vacuolization to form autophagosome, phagocytosis and subsequent lysosomal degradation. (3) Type III or necrosis, manifesting with distinctive morphology, is different from type I and type II cell death. Its morphological changes contain organelle and plasma membrane rupture, finalizing with disposal of cell corpses absent of evident phagocytic and lysosomal involvement. (4) Type IV or entosis, displaying “cell-in-cell” cytological features in which cell winners engulf and kill cell losers (Martins et al., 2017; Tang et al., 2019a) (Figure 1). Biopsy showed that the apoptosis of a diabetic heart was 85 times higher than that of the nondiabetic heart, indicating that cardiomyocytes in diabetes were sensitive to suffer cell death (Cai and Kang, 2003). To understand the detailed mechanism of cell death in cardiomyocytes is beneficial to propose novel and ideal strategies for DCM.

MECHANISMS AND IMPLICATIONS OF CELL DEATH IN DCM

Apoptosis in DCM

Apoptosis is also called programmed cell death (PCD), which is the fastest form of cell death. Proteolytic cascade induced by caspases is the key biochemical characteristic of apoptosis. Caspases with an inactive proenzyme form are widely expressed in cardiomyocytes. Immediately after activation, a protease cascade is initiated by other procaspases depending on their proteolytic activity. This proteolytic cascade amplifies apoptotic pathways and finally results in rapid and irreversible cell death (Huang et al., 2019). There are two main pathways, including extrinsic apoptotic pathway and intrinsic apoptotic pathway, involved in the mechanisms of apoptosis. The extrinsic apoptotic pathway is also known as death receptor pathway, during which multiple death ligands, such as tumor necrosis factor-α (TNF-α) and Fas, bind to their homologous receptors to trigger cell death. Then, caspase 8 and caspase 9 are initiated to subsequently activate executioner caspases (caspase 3, caspase 6, and caspase 7), and finally result in apoptosis. The intrinsic pathway is known as mitochondrial pathway. During intrinsic pathway, a group of proteins called the complex permeability transition pore (CPTP) form a megapore and cover the inner and outer membranes of the mitochondria. Mitochondrial proteins such as cytochrome C (Cyto C), high temperature requirement protein A2 (HtrA2)/Omi, and second mitochondria-derived activator of caspases/direct inhibitors of apoptosis-binding protein with low pI (Smac/Diablo) are released into the cytosol. Then, Cyto C combines with apoptosis protease activating factor-1 (Apaf-1) to form a complex named “apoptosome”, which serves as a platform for the cleavage and activation of downstream caspases (D’Arcy, 2019). Activation of caspases ultimately leads to cell dismantling (Figure 2).

A great number of studies have demonstrated that long-term hyperglycemia in diabetic patients induces cardiomyocyte apoptosis (Joubert et al., 2019). Cardiomyocyte apoptosis leads to cell loss to decrease cardiac contractile function and to ultimately promote cardiac remodeling (Hu et al., 2017). It has been proven that astragalus polysaccharides (APS) protected HG
**Mechanism of apoptosis in DCM.** Both extrinsic apoptotic pathway and intrinsic apoptotic pathway are involved in DCM. Extrinsic pathway: long-term hyperglycemia triggers TNF-α to bind to TNF-α receptor (TNFR), initiates caspase 8 and subsequent caspase 3 activation, and finally results in apoptosis in the cardiomyocytes. Intrinsic pathway: Cyto C released into the cytosol combines with Apaf-1 to form a complex serving as a platform for caspase 9 and caspase 3 activation. Apoptosis induces cardiomyocyte cell loss to ultimately promote DCM.
associated with enhanced autophagy in the myocardium of diabetic Goto-Kakizaki rats (Liu et al., 2014). Hence, determining whether autophagy is adaptive or maladaptive is critical for the execution of efficient therapeutic treatment for DCM.

Necrosis in DCM
Necrotic cell death contains a great diversity of cell death processes. Necrosis may occur in the case of extensive damage such as high temperature and mechanical stress, leading to cell integrity destruction (Marunouchi and Tannaka, 2015). In this case, necrosis is passive and does not require any specific signaling pathways. Another type of necrosis, called secondary necrosis, occurs at the late stage of apoptosis or autophagy when dead cells can’t be removed by phagocytosis. Secondary necrosis is regarded as independent of initial signal events such as apoptosis or autophagy (Wu et al., 2018b). However, necrosis can also be a result of signaling cascade. Early study showed that diabetes increased necrosis by fourfold in cardiomyocytes (Frustaci et al., 2000).

Necroptosis is the best-characterized form of programmed necrosis, showing features of both apoptosis and necrosis (Tang et al., 2019a). The pathway of necroptosis activation after high glucose stimulation is mediated by ligand to death receptors such as tumor necrosis factor receptor 1 (TNFR1) and Fas receptors. Damage-associated molecular patterns (DAMPs), nucleotide-binding, and oligomerization domain (NOD)-like receptors (NLRs) ripoptosome, and protein kinase R (PKR) complexes also trigger necroptosis. TNFR1-mediated necroptosis is the most thoroughly studied pathway to activate necroptosis. After TNF-α binds to TNFR1, there are three possible divergent functions including cell survival, apoptosis, or necrosis via different signaling complexes. Complex I is prosurvival and Complex Ila is proapoptotic. Complex Iib formation results in necroptosis (Galluzzi et al., 2018). Receptor-interacting protein 3 (RIP3) is regarded as a critical regulator of necroptosis. RIP3 regulates necroptosis on a RIP1-dependent manner in diabetes. Once recruited by RIP1 after HG exposure, RIP3 is activated by auto-phosphorylation to promote mixed lineage kinase domain like protein (MLKL) phosphorylation. Then, MLKL oligomerizes and translocates to the cell membrane, interacts with phosphatidylinositol lipids and cardiolipin to result in membrane permeabilization (Gupta et al., 2018). Calmodulin-dependent protein kinase II (CaMKII) is a newly found RIP3 substrate to induce necroptosis. CaMKII is abundant in the myocardium and is inactivated under normal conditions. CaMKII phosphorylation by intracellular Ca^{2+} or RIP1 facilitates necroptosis (Nomura et al., 2014). Meanwhile, HG also increases ROS to activate CaMKII by oxidation (Feng and Anderson, 2017). Altogether, the above pathological changes trigger the opening of mitochondrial permeability transition pore (mPTP), which is involved in the final pathway of necroptosis (Figure 3). Some research found that knockdown of cyclophilin D (CypD), a protein that increases mPTP opening probability, protected against RIP3-induced cardiomyocyte necrosis (Zhang et al., 2016). RIP3 up-regulation increased phosphoglycerate mutase 5 (PGAM5) expression, enhanced CypD phosphorylation, and finally resulted in mPTP opening. Excessive opening of the mPTP also amplified the death signal and ultimately led to necroptosis in the endothelial cells (Zhou et al., 2018a).

It was observed that when H9C2 cells were exposed to high glucose, cell viability, the activity of ALDH2 mRNA or protein expression were suppressed, while ROS levels as well as RIP1, RIP3, and MLKL expression were enhanced. Necrostain-1 (Nec-1, a specific inhibitor of necroptosis) or Alda-1 (the ALDH2 activator) attenuated HG-induced down-regulation of ALDH2 and enhancement of RIP1, RIP3 and MLKL. In general, ALDH2 activation protected against HG-induced H9C2 cell injury, partly by inhibiting necroptosis (Fang et al., 2018). Another study showed that HG markedly increased the expression of Toll-like receptor 4 (TLR4), which was attenuated by NAC, a ROS scavenger. HG also significantly increased the expression of RIP3, which was ameliorated by TAK-242 (an inhibitor of TLR4) or Nec-1. In addition, NAC mitigated HG-induced RIP3 expression up-regulation. More importantly, DZ (a mitochondrial K_{ATP} channel opener) and Pin (a non-selective K_{ATP} channel opener) reduced the increased levels of TLR4 and RIP3 induced by HG. Taken together, the opening of K_{ATP} channels inhibited necroptosis through ROS-TLR4 pathway to protect H9C2 cells against HG-induced damage (Liang et al., 2017). Our recent research revealed that inhibitor 1 of protein phosphatase 1 (1PP1) over-expression alleviated CaMKIIα alternative splicing disorder, suppressed ROS overproduction, inhibited CaMKII oxidation, suppressed necroptosis, and ultimately alleviated high glucose-induced cardiomyocyte injury (Sun et al., 2019a).

Pyroptosis is another novel form of programmed necrosis (Robinson et al., 2019). It is responsible for cellular lysis and extracellular release of proinflammatory cytokines such as interleukin-1β (IL-1β) and interleukin-18 (IL-18) by HG stimulation (Zeng et al., 2019). There are two different pathways including the canonical pathway and noncanonical pathway in pyroptosis. In the canonical pathway, cytoplasmic multiprotein complexes, named inflammasome, consist of the nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family (including NLRP3, NLRP1, NLRC4, NLRP9 and NLRP6), the pyrin and HIN domain (PYHIN) protein families (absent in melanoma 2, AIM2), and pyrin proteins. Distinct inflammasomes are recognized by pathogen-associated molecular patterns (PAMPs) or DAMPs to activate caspase 1, leading to pyroptosis (Lee et al., 2019). In the noncanonical pathway, bacterial lipopolysaccharide (LPS) is delivered to the cytosol to activate caspase 11. Activated caspase 11 directly induces pyroptosis. The cleaved caspase 11 also activates the Gasdermin-D (GSDMD) to form a membrane pore. Meanwhile, the NLRP3 inflammasome is activated by the N-terminal fragment of GSDMD (GSDMD-N) to trigger pyroptosis via the canonical pathway (Zhao et al., 2019).

Emerging evidence has verified that pyroptosis is involved in the progression of DCM. Jeyabal et al. found that NLRP3, caspase 1, and ELAV-like RNA binding protein 1 (ELAVL1,
also called Hu-Antigen, HuR) expression in both human diabetic heart and HG-exposed human ventricular cardiomyocytes were augmented. Further study identified HuR as a direct target of miR-9. MiR-9 mimic transfection reduced HuR, caspase 1, and IL-1β expression to inhibit HG-induced pyroptosis in human cardiomyocytes. This study highlighted that targeting miR-9/HuR would play a therapeutic role in cell death during DCM (Jeyabal et al., 2016). Recently, Wang et al. found that absent in melanoma 2 (AIM2) in inflamasome, caspase-1, IL-1β, and GSDMD were elevated in the myocardium of diabetes mellitus. ROS was also increased in H9C2 cells with HG stimulation. AIM2-shRNA reduced caspase 1 and IL-1β expression, suppressed GSDMD-N level and attenuated HG-induced pyroptosis. Furthermore, AIM2 level dropped significantly if ROS was inhibited. This study indicated that AIM2 inhibition is beneficial to attenuate diabetic cardiomyopathy via alleviating GSDMD-N-related pyroptosis (Wang et al., 2019d) (Figure 4). These findings confirmed the distinctive role of pyroptosis in DCM. To inhibit the pyroptosis signaling pathways is to capably expand the potential therapeutic targets for DCM treatment.

Other Types of Cell Death in DCM

Entosis seems fundamentally different from most typical forms of cell death due to the requirement of engulfing cells to execute. Entosis is mediated by cellular engulfment via E-cadherin, α-catenin, Rho family of GTPases, and rho-associated kinase (ROCK). Entosis is identified as an essential mechanism of embryo implantation (Li et al., 2015), which plays pro- and antitumorigenic roles in cancer. However, no data regarding the role of entosis in cardiovascular diseases has been found until now, and the possible mechanisms were incompletely defined.

Ferroptosis is an iron- and lipotoxicity-dependent form of regulated cell death (RCD). It is characterized by small mitochondria with reduced crista and condensed or ruptured outer membrane (Baba et al., 2018). Acyl-CoA synthetase long-chain family member 4 (ACSL4), lysophosphatidylcholine acyltransferase 3 (LPCAT3), and arachidonate lipoxygenase (ALOXs) pathways mediate the oxidation of polyunsaturated fatty acids, which is necessary for the lipid toxicity of ferroptosis (Dixon et al., 2015; Chu et al., 2019; Xiao et al., 2019). ACSL4 up-regulation is the marker of ferroptosis (Doll et al., 2017). Decreasing antioxidant glutathione or inhibiting glutathione peroxidases inevitably enhanced ROS formation by erastin, a cell-permeable ferroptosis activator (Yang et al., 2014). Ferroptosis has been reported to be regulated during drug therapy; whether it participates in the pathology of DCM is unclear. In consideration of ROS formation promoting ferroptosis, there is a serious possibility that ferroptosis is involved in DCM.

Parthanatos is a poly ADP-ribose (PAR) polymerase-1 (PARP-1)-dependent type of cell death, which is activated by oxidative stress-induced DNA damage (Wang et al., 2019e). Neither apoptotic body formation nor DNA fragmentation is
observed during parthanatos. Parthanatos does not produce cell swelling or lysosomal degradation. Energy exhaustion and apoptosis-inducing factor (AIF) release from the mitochondria mediated by PAR or calpain are possibly the main mechanisms of parthanatos. Parthanatos has been reported to be involved in cardiovascular diseases, renal diseases, diabetes, and neurodegeneration (Linkermann, 2016; Barany et al., 2017; Kam et al., 2018; Aizawa et al., 2019; Li et al., 2019d). PARP inhibitor L-2286 prevented cardiac remodeling, improved systolic function, and delayed the development of heart failure (Bartha et al., 2009). Thus, PARP inhibition may have the potential ability to attenuate DCM.

Netotic cell death is a form of RCD driven by neutrophil extracellular traps (NETs), which is regulated by NADPH oxidase-mediated ROS production and histone citrullination. NET formation and release, or NETosis relies on ROS production, autophagy, granular enzyme release, and translocation from the cytosol to the nucleus. Histone citrullination eventually leads to chromatin depletion, nuclear membrane destruction, and chromatin fiber release (Hemmers et al., 2011; Mitroulis et al., 2011; Tang et al., 2019a). Efforts could be endeavored to explore the mechanisms of NETosis underlying DCM, thereby providing new therapeutic policies for DCM.

Lysosomal-dependent cell death (LCD), also known as lysosomal cell death, is a form of regulated cell death mediated by intralysosomal components or ion translocation resulting from lysosomal membrane permeabilization (LMP) to amplify or initiate cell death during apoptosis, autophagy, and ferroptosis (Wang et al., 2018). Although the relation between LCD and DCM was easy to be deduced, further studies are still indispensable.

Alkaliptosis is driven by intracellular alkalization. It is known that down-regulation of inhibitor of nuclear factor kappa B kinase subunit beta (IKBKB) and nuclear factor-κB (NF-κB) induces alkaliptosis (Song et al., 2018). The pathological significance of alkaliptosis in human diseases remains complicated, and the main signals of alkaliptosis need to be illuminated in the future.

Oxietosis is a novel caspase-independent RCD induced by ROS, which is mediated through Kelch-like ECH-associated protein 1 (KEAP1)/PGAM5/apoptosis-inducing factor 1 mitochondrial (AIFM1) pathway (Scaturo and Pichlmair, 2018). Since ROS accumulation links to multiple physiological and pathological processes, it is very likely that oxietosis promotes the development of several diseases including DCM.

Different types of cell death are mediated by distinct but overlapping central pathways. One study found that autophagy was inhibited but apoptosis was amplified in diabetic heart (Xing et al., 2019). Elevated bax/bcl-2 ratio, increased cleaved caspase 3 level, augmented NLRP3, cleaved caspase 1, IL-1β, and cleaved GSDMD expression along with increased ROS production were detected in the myocardium of db/db mice, indicating the involvement of apoptosis and pyroptosis in DCM (Xue et al., 2019). Altogether, several types of cell death may coexist in the occurrence and development of DCM. Moreover, apoptosis, necrosis, necroptosis, pyroptosis, autophagy, or ferroptosis might act simultaneously during DCM. Inhibition of one kind of cell death may possibly promote other types of cell death and cause compensation, which eventually increases the complexity of pathogenesis about DCM and enhance difficulty of treatment for DCM.

### CONCLUSION AND PROSPECTIVE

Current therapeutic strategies for DCM involve insulin and insulin-secreting agents, oral antihyperglycemic medication, β-blockers, angiotensin converting enzyme inhibitors, and pathological processes need to be clarified, and new therapeutic policies need to be developed for DCM.

### TABLE 1 | Drugs or compounds against cell death in diabetic cardiomyopathy.

| Drugs or compounds | Targets of cell death type | Effects | References |
|--------------------|--------------------------|---------|-----------|
| Astragalus          | apoptosis                | oxidative stress↑, antioxidant capacity↑ | (Sun et al., 2019b) |
| Polysaccharides     | apoptosis                | bax/bcl-2↓, cleaved caspase 3↑ | (Zou et al., 2019b) |
| Catalpol            | autophagy                | LC3II/LC3↑ | (Fediantsis et al., 2018) |
| Crocin             | autophagy                | cytochrome C1, bcl-2↑ | (Yao et al., 2018) |
| Curcumin           | autophagy                | cleaved caspase 3↑, cleaved caspase 9↑ | (Oh et al., 2019) |
| Dexametomidine      | autophagy                | LC3II/LC3↑, p62↑, ATG7↑ | (Wu et al., 2017) |
| Dihydrorictinatin   | autophagy                | LC3II/LC3↑, p62↑, ATG7↑ | (Wei et al., 2017) |
| 1,25-Dihydroxyvitamin-D3 | autophagy                | LC3II/LC3↑ | (Xue et al., 2019) |
| Empagliflozin       | apoptosis                | bax/bcl-2↓, cleaved caspase 3↑ | (Li et al., 2019b) |
| NaHS               | pyroptosis               | NLRP3↑, caspase 11↑, IL-1β↑, cleaved GSDMD↑, full-length GSDMD↑ | (Zou et al., 2019a) |
| Nicorandil         | apoptosis                | bax/bcl-2↓, cleaved caspase 3↑ | (Liang et al., 2016) |
| Notoginsenoside     | apoptosis                | mitochondrial membrane depolarization↑, ROS↓ | (Wang et al., 2019c) |
| Okicadric acid      | apoptosis                | bax/bcl-2↓, cleaved caspase 3↑ | (Ren et al., 2016) |
| Pabociclib         | apoptosis                | bax/bcl-2↓, cleaved caspase 3↑ | (Zou et al., 2019a) |
| Plicamycin         | apoptosis                | bax/bcl-2↓, cleaved caspase 3↑ | (Li et al., 2019a) |
| Resveratrol        | apoptosis                | cleaved caspase 3↑, cleaved caspase 9↑ | (Zou et al., 2019a) |
| Sitaglitin         | autophagy                | LC3II/LC3↑, p62↑ | (Zou et al., 2018b) |
| Sophoracine        | autophagy                | LC3II/LC3↑, p62↑ | (Zou et al., 2019a) |
| Vasin              | autophagy                | LC3II/LC3↑, p62↑ | (Li et al., 2019c) |
angiotensin II receptor antagonists, calcium channel antagonists, and hydroxymethylglutaryl CoA reductase inhibitors. However, the above synthetic drugs have many adverse effects including gastrointestinal disturbances, hepatotoxicity, abdominal pain, genitourinary tract infection, and so on (Parim et al., 2019). As cell death is a determinant pathological fate in DCM, the executors of cell death signal pathways are obviously potential therapeutic targets for DCM. Many drugs or compounds are being used in or under clinical trials. Take some examples: Metformin, a common antidiabetic drug, activates AMPK and improves autophagy via inhibiting the mTOR pathway and alleviating pyroptosis (Yang et al., 2019). Crocin, a carotenoid extracted from saffron, improves the deteriorated cardiac function in diabetic animals by inhibiting apoptosis and normalizing autophagy (Feidantsis et al., 2018). Protein phosphatase 2A (PP2A) is a central cardiac phosphatase that regulates the functions of diverse myocytes through target molecules. Okadaic acid (OA), an inhibitor of PP2A, inhibited apoptosis of experimental diabetic mellitus-related cardiomyopathy (Guan et al., 2019). These researches will improve the understanding of cell death in DCM and set the stage for novel therapies in future. Several drug or compounds targeting the signals in cell death have been used in clinics or experiments for DCM (Table 1).

Taken together, cell death is the terminal pathway of cardiomyocytes during DCM. Although upstream signal regulation seems to attenuate cell death effectively, it may produce a series of nonspecific effects. Targeting cell death directly may be more practicable than its upstream signaling pathway.

**AUTHOR CONTRIBUTIONS**

YC researched documents and wrote the manuscript. YH, XL, IA, and WZ revised the manuscript. GM designed the study and reviewed the manuscript. All authors approved the final version of the manuscript.

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**REFERENCES**

Adingupu, D. D., Gopel, S. O., Gronros, J., Behrendt, M., Sotak, M., Milliotis, T., et al. (2019). SGLT2 inhibition with empagliflozin improves coronary microvascular function and cardiac contractility in prediabetic ob/ob(-/-) mice. *Cardiovasc. Diabetol.* 18, 16. doi: 10.1186/s12933-019-0820-6

Aitawa, S., Brar, G., and Tsukamoto, H. (2019). Cell death and liver disease. *Gut.* 14, 20–29. doi: 10.1050/gnl18486

Baba, Y., Higa, J. K., Shimada, B. K., Horinouchi, K. M., Suhara, T., Kobayashi, M., et al. (2018). Protective effects of the mechanistic target of rapamycin against excess iron and ferroptosis in cardiomyocytes. *Am. J. Physiol. Heart Circ. Physiol.* 314, H659–H668. doi: 10.1152/ajpheart.00452.2017

Barany, T., Simon, A., Szabo, G., Benko, R., Mezei, Z., Molnar, L., et al. (2017). Oxidative stress-related parthanatos of circulating mononuclear leukocytes in heart failure. *Oxid. Med. Cell. Longev.* 2017, 1249614. doi: 10.1152/2017/1249614

Bartha, E., Solti, I., Kereskai, L., Lantos, J., Plozer, E., Magyar, K., et al. (2017). PARP inhibition delays transition of hypertensive cardiopathy to heart failure in spontaneously hypertensive rats. *Cardiovasc. Res.* 103, 501–510. doi: 10.1093/cvr/csv144

Bhattacharya, D., Mukhopadhayay, M., Bhattacharyya, M., and Karmakar, P. (2018). Is autophagy associated with diabetes mellitus and its complications? A review. *EXCLI J.* 17, 709–720. doi: 10.17197/excli2018-1553

Bootman, M. D., Chehab, T., Bultynck, G., Parys, J. B., and Rietdorf, K. (2018). The regulation of autophagy by calcium signals: Do we have a consensus? *Cell Calcium.* 70, 32–46. doi: 10.1016/j.ceca.2017.08.005

Cai, L., and Kang, Y. J. (2003). Cell death and diabetic cardiomyopathy. *Cardiovasc. Toxicol.* 3, 219–228. doi: 10.1085/CT:3:3219

Cao, H., Chen, T., and Shi, Y. (2015). Glycation of human serum albumin in diabetes: impacts on the structure and function. *Curr. Med. Chem.* 22, 4–13. doi: 10.2174/09298671321666140912155738

Chen, X. F., Li, X. L., Yang, M., Song, Y., and Zhang, Y. (2018). Osteoprotective effects of salidroside in ovariectomized mice and diabetic mice. *Eur. J. Pharmacol.* 819, 281–288. doi: 10.1016/j.ejphar.2017.12.025

Chu, B., Kon, N., Chen, D., Li, T., Liu, T., Jiang, L., et al. (2019). ALOX12 is required for p53-mediated tumour suppression through a distinct ferroptosis pathway. *Nat. Cell Biol.* 21, 579–591. doi: 10.1038/s41556-019-0305-6

D’Arcy, M. S. (2019). Cell death: a review of the major forms of apoptosis, necrosis and autophagy. *Cell Biol. Int.* 43, 582–592. doi: 10.1002/cbin.11137

Dixon, S. J., Winter, G. E., Musavi, L. S., Lee, E. D., Snijder, B., Rebsamen, M., et al. (2015). Human haploid cell genetics reveals roles for lipid metabolism genes in nonapoptotic cell death. *ACS Chem. Biol.* 10, 1604–1609. doi: 10.1021/acschembio.5b00245

Doll, S., Proneth, B., Tyurina, Y. Y., Panzilius, E., Kobayashi, S., Ingold, I., et al. (2017). ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat. Chem. Biol.* 13, 91–98. doi: 10.1038/nchembio.2239

Fang, T., Cao, R., Wang, W., Ye, H., Shen, L., Li, Z., et al. (2018). Alterations in necroptosis during ALDH2-mediated protection against high glucoseinduced H9c2 cardiac cell injury. *Mol. Med. Rep.* 18, 2807–2815. doi: 10.3892/mmr.2018.9269

Feidantsis, K., Mellidis, K., Galatou, E., Sinakos, Z., and Lazou, A. (2018). Treatment with crocin improves cardiac dysfunction by normalizing autophagy and inhibiting apoptosis in STZ-induced diabetic cardiomyopathy. *Nutr. Metab. Cardiovasc. Dis.* 28, 952–961. doi: 10.1016/j.numecd.2018.06.005

Feng, N., and Anderson, M. E. (2017). CaMKII is a nodal signal for multiple programmed cell death pathways in heart. *J. Mol. Cell. Cardiol.* 103, 102–109. doi: 10.1016/j.yjmcc.2016.12.007

Fustaci, A., Kajstura, J., Chimenti, C., Jakoniuk, I., Leri, A., Maseri, A., et al. (2000). Myocardial cell death in human diabetes. *Circ. Res.* 87, 1123–1132. doi: 10.1161/01.RES.87.12.1123

Galluzzi, L., Vitale, I., Aaronson, S. A., Abrams, J. M., Adam, D., Agostinis, P., et al. (2018). Molecular mechanisms of cell death: recommendations of the
Sun, L., Chen, Y., Luo, H., Xu, M., Meng, G., and Zhang, W. (2019a). Ca(2 +)-calmodulin-dependent protein kinase II regulation by inhibitor 1 of protein phosphatase 1 alleviates necroptosis in high glucose-induced cardiomyocytes injury. Biochem. Pharmacol. 163, 194–205. doi: 10.1016/j.bcp.2019.02.022

Sun, Q., Wu, X., Wang, H., Chen, W., Zhao, X., Yang, Y., et al. (2019b). Protective effects of astagalos polysaccharides on oxidative stress in high glucose-induced ORS D262 Silenced H9C2 cells based on PCR Array Analysis. Diabetes Metab. Syndr. Obes. 12, 2209–2220. doi: 10.2147/DMSO.S228351

Sun, S., Yang, S., An, N., Wang, G., Xu, Q., Liu, J., et al. (2019c). Astagalos polysaccharides inhibits cardiomyocyte apoptosis during diabetic cardiomyopathy via the endoplasmic reticulum stress pathway. J. Ethnopharmacol. 238, 111857. doi: 10.1016/j.jep.2019.111857

Tan, X., Hu, L., Shu, Z., Chen, L., Li, X., Du, M., et al. (2019). Role of CCR2 in the development of streptozotocin-treated diabetic cardiomyopathy. Diabetes 68, 2063–2073. doi: 10.2337/db18-1231

Tang, D., Kang, R., Berge, T. V., Vandenabeele, P., and Kroemer, G. (2019a). The molecular machinery of regulated cell death. Cell Res. 29, 347–364. doi: 10.1038/s41422-019-0164-5

Tang, S. G., Liu, X. Y., Wang, S. P., Wang, H. H., Jovanovic, A., and Tan, W. (2019b). Mitochondria regulates autophagy in streptozotocin-induced diabetes. Diabetologia 62, 3292–3306. doi: 10.1007/s00125-019-04769-0

Wang, F., Gomez-Sintes, R., and Boya, P. (2018). Lysosomal membrane permeabilization and cell death. Frontiers in Pharmacology | www.frontiersin.org February 2020 | Volume 11 | Article 429

Wang, J., Liu, H., Li, N., Zhang, Q., and Zhang, H. (2014). The protective effect of curcumin in the development of streptozotocin-treated diabetic cardiomyopathy. Diabetes 63, 1026–1032. doi: 10.2373/dlbh1231

Wang, X., Pan, J., Liu, H., Zhang, M., Li, X., Tian, J., et al. (2019c). Nicorandil reducing ryanodine receptor 2 activity. Cell Death in DCM

Wang, L., Yuan, D., Zheng, J., Wu, X., Wang, J., Liu, X., et al. (2019b). Chikusetsu polysaccharides inhibits cardiom yocyte apoptosis during diabetic cardiomyopathy via sGC-GMP-PKG pathway in type 2 diabetes mice. Clin. Sci. (Lond) 133, 1705–1720. doi: 10.1042/CS20190585

Wang, Y., Liang, Z. Q., Gu, Z. L., and Qin, Z. H. (2005). Molecular mechanism and regulation of autophagy. Acta Pharmacol. Sin. 26, 1421–1434. doi: 10.1111/j.1745-7254.2005.00235.x

Wang, Y. S., SriRamaratnam, R., Welsch, M. E., Shimada, K., Skouta, R., Viswanathan, V. S., et al. (2014). Regulation of ferroptotic cancer cell death by GPX4. Cell 156, 317–331. doi: 10.1016/j.cell.2013.12.010

Wang, Y., Qin, Y., Wang, Y., Meng, S., Xian, H., Che, H., et al. (2019). Metformin inhibits the NLRP3 Inflammasome via AMPK/mTOR-dependent effects in diabetic cardiomyopathy. Int. J. Biol. Sci. 15, 1010–1019. doi: 10.7150/ijbs.29680

Zeng, C., Wang, R., and Tan, H. (2019). Role of pyroptosis in cardiovascular diseases and its therapeutic implications. Int. J. Biol. Sci. 15, 1345–1357. doi: 10.7150/ijbs.33568

Zhang, T., Zeng, Z., Yuan, X., Lin, L., Liu, Y., An, N., et al. (2019b). Empagliflozin prevents cardiomyopathy via sGC-GMP-PKG pathway in type 2 diabetes mice. Brain Res. 1720. doi: 10.1016/j.brainres.2019.04.004

Zhao, Z., Guo, L., Shi, W., and Zuo, W. (2019). Role of pyroptosis in cardiovascular diseases and its therapeutic implications. Int. J. Biol. Sci. 15, 1345–1357. doi: 10.7150/ijbs.33568

Zhou, H., Li, D., Zhu, P., Ma, Q., Toan, S., Wang, J., et al. (2018a). Inhibitory effect of BDNF on AMPK activation and alleviating apoptosis. J. Mol. Cell. Cardiol. 124, 26–34. doi: 10.1016/j.yjmcc.2018.04.004

Zhou, H., Li, D., Zhu, P., Ma, Q., Toan, S., Wang, J., et al. (2018b). Nognigeneside R1 protects against diabetic cardiomyopathy through activating estrogen receptor alpha and its downstream signaling. Front. Pharmacol. 9, 1227. doi: 10.3389/fphar.2018.01227

Zhao, Z., Guo, L., Shi, W., and Zuo, W. (2019). Role of pyroptosis in cardiovascular disease. Cell Prolif. 52, e12563. doi: 10.1111/cpr.12563

Zheng, C., Wang, R., and Tan, H. (2019). Role of pyroptosis in cardiovascular diseases and its therapeutic implications. Int. J. Biol. Sci. 15, 1345–1357. doi: 10.7150/ijbs.33568

Zhang, T., Zeng, Z., Yuan, X., Lin, L., Liu, Y., An, N., et al. (2019b). Empagliflozin prevents cardiomyopathy via sGC-GMP-PKG pathway in type 2 diabetes mice. Brain Res. 1720. doi: 10.1016/j.brainres.2019.04.004

Zhou, H., Li, D., Zhu, P., Ma, Q., Toan, S., Wang, J., et al. (2018a). Inhibitory effect of BDNF on AMPK activation and alleviating apoptosis. J. Mol. Cell. Cardiol. 124, 26–34. doi: 10.1016/j.yjmcc.2018.04.004

Zhou, H., Li, D., Zhu, P., Ma, Q., Toan, S., Wang, J., et al. (2018b). Nognigeneside R1 protects against diabetic cardiomyopathy through activating estrogen receptor alpha and its downstream signaling. Front. Pharmacol. 9, 1227. doi: 10.3389/fphar.2018.01227

Zhou, H., Li, D., Zhu, P., Ma, Q., Toan, S., Wang, J., et al. (2018a). Inhibitory effect of melatonin on necroptosis via repressing the RIK3-PGAM5–CypD–mPTP pathway attenuates cardiac microvascular ischemia-reperfusion injury. J. Pineal. Res. 65, e12503. doi: 10.1111/jpr.12503

Zhou, Y., Wang, H., Man, F., Guo, Z., Xu, J., Yan, W., et al. (2018b). Statin protects cardiac function by reducing nitrosative stress and promoting autophagy in Zucker Diabetic Fatty (ZDF) rats. Cardiovasc. Drugs Ther. 32, 541–552. doi: 10.1007/s10557-018-6831-9

Zou, F., Wang, L., Liu, H., Wang, W., Hu, L., Xiong, X., et al. (2019a). Sophoracin suppresses NF-kappaB-mediated inflammation both in vitro and in vivo and inhibits diabetic cardiomyopathy. Front. Pharmacol. 10, 1219. doi: 10.3389/fphar.2019.01219

Zou, G., Zhong, W., Wu, F., Wang, X., and Liu, L. (2019b). Catalpol attenuates cardiomyocyte apoptosis in diabetic cardiomyopathy via Neat1/miR-140-5p/HDAC4 axis. Biochimie 165, 90–99. doi: 10.1016/j.biochi.2019.05.005

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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