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Review Article

THE RELATIONSHIP BETWEEN PYROPTOSIS AND ACS

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ABSTRACT

The functioning of the heart is determined by the extracellular matrix composing it and the dynamic equilibrium of different types of cells. Different cardiovascular diseases such as acute coronary syndrome are always accompanied by the death of cells and chronic/acute inflammatory reactions. Caspase-dependent pyroptosis is categorized by path activation, resulting in NOD-like receptors, particularly the inflammasome of the NLRP3 and its downstream inflammatory effector factors interleukin. The findings of this study have resulted in the growth of therapeutic approaches based on pyroptosis regulation. The paper discusses the relationship between pyroptosis and acute coronary syndrome.

Keywords: pyroptosis, acute coronary syndrome, inflammasome, caspase

INTRODUCTION

The death of cells is crucial to maintaining basic biological functions and tissue homeostasis, and its alterations have important implications in the pathology of diseases. Since the death of cells was initially described in the mid-20th century, numerous types of cell death are defined depending on variances in biochemical and morphological characteristics (Place & Kanneganti, 2019). The function of the cardiovascular system and the maintenance of the normal structure needs a balance between the formation of cells and death in the organs and tissues of the cardiovascular system. Excessive death of cells (including pyroptosis) often results in organ and tissue dysfunction (Paradies et al., 2018).

Pyroptosis is important in the pathogenesis of different cardiovascular diseases and is a highly regulated process of cell death. The process is considered as a target for therapeutic intervention to curb cardiovascular diseases. This study provides an overview of pyroptosis's functional role and evidence in acute coronary syndrome (ACS).

Overview of Pyroptosis:

Pyroptosis is a form of programmed death of cells that is supplemented by an inflammatory response. Programmed death of cells is defined as the autonomous, ordered cell death controlled by genes to maintain a balance in the human body (homeostasis). On the other hand, non-programmed death of cells is defined as the necrosis of cells involving passive cells' death if exposed to chemical or physical stimuli in the surrounding (Qin et al., 2017).

Moreover, programmed death of cells can always be prevented by cellular signal transduction inhibitors, while non-programmed death of cells cannot. Pyroptosis is activated by different pathological stimuli, including inflammation, hyperglycemia, oxidative stress, and controlling various microbial infections (Qiao et al., 2019).

Pyroptosis is classified by rapid disruption of the plasma membrane followed by the release of pro-inflammatory mediators and cellular contents such as IL-1β and IL-18. Unlike many cytokines, IL-1β and IL-18 are not removed by the classical endoplasmic reticulum. Rather, they are produced as inactive biological precursor proteins cleaved before their secretion as bioactive cytokines (Qiu et al., 2019). At first, IL-1β is synthesized as a molecule of active precursor that must be cleaved at the position of amino acids by caspase-1 to produce IL-1β p17 that is actively mature.

Nevertheless, mature IL-1β is considered a pro-inflammatory mediator recruiting innate cells immune to sites of infection and modulating adaptive immune cells (Qiu et al., 2019). The same mature IL-1β is essential for interferon-y production and cytolytic activity potentiation of T- cells and natural killer cells (Qiu et al., 2019). It may also polarize the T-cells towards Th2 or Th1 profiles together with other cytokines. Pyroptosis primarily involves the canonical caspase-1 dependence pathway and the non-canonical pathway of caspase-4,5 and caspase-11.

The inflammasomes are triggered by the cells such as NLRP3, which is absent in pyrin or melanoma 2 through pathogen-linked molecular patterns actions as well as molecular patterns that are associated with

danger under hyperlipidemia stimulation, inflammation, and HG (Zheng & Li, 2020). After the NLRP3 activation, the NLRP3 N-terminal pyrin domain acts as a scaffold to nucleate speck-like protein that is connected with apoptosis which contains caspase recruitment, and activation domain that also contains a caspase recruitment and activation domain as well as a pyrin domain (Reboredo-Rodríguez et al., 2018).

Through the pyrin domain, there is the interaction of the sensor molecules by the ASC with pro-caspase-1 that initiates its self-cleavage to form a mature body of caspase-1. Additionally, the triggered caspase-1 then recognizes the inactive precursors of IL-1β and IL-18 and converts them into inflammatory cytokines that are mature (Ruan et al., 2018). The formation of the pores of the membrane thus, promotes inflammatory factors' release, swelling of cells, and pyroptosis.

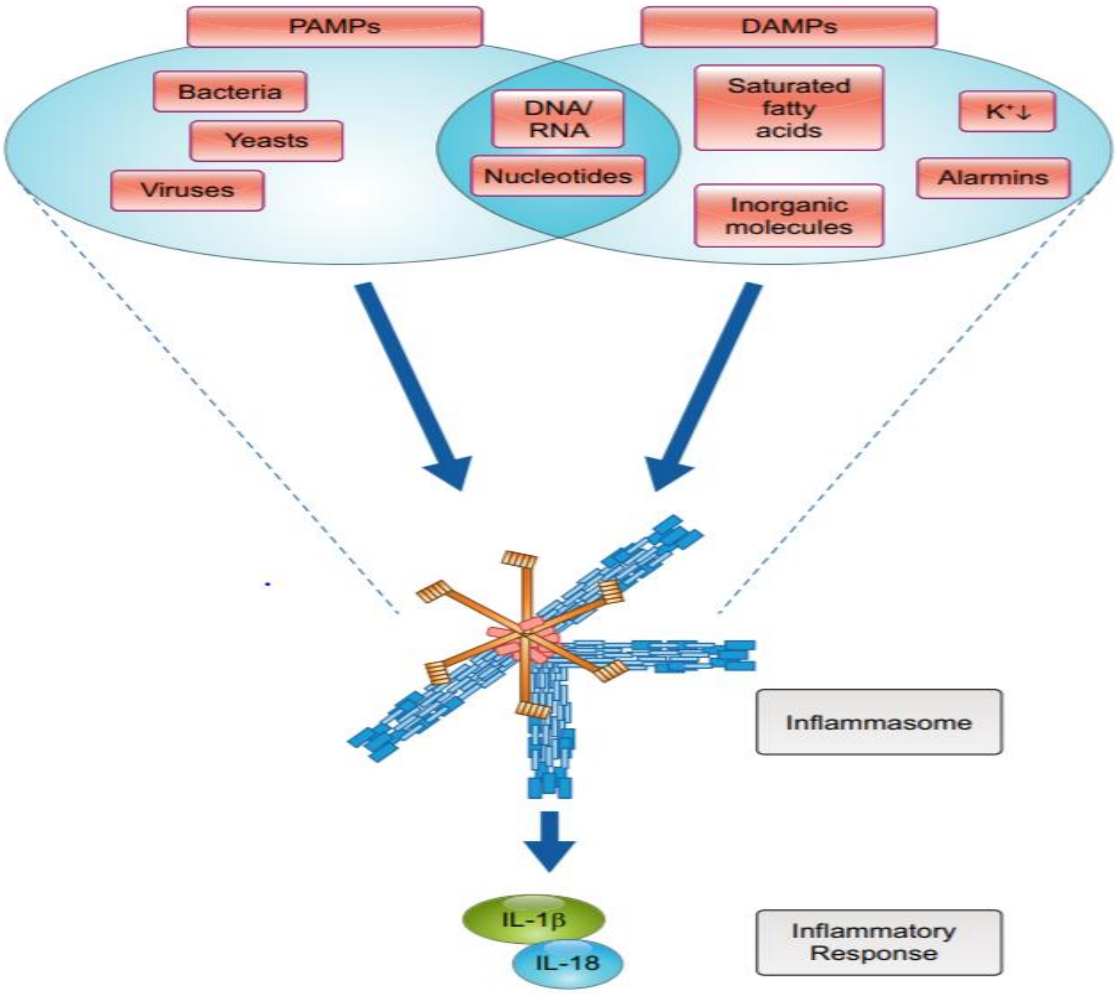

Figure 1: Inflammasomes reaction to danger-associated molecule presence triggering a certain cytokine pathway.

In the non-canonical route, the lipopolysaccharide component of the Gram-negative bacterial cell wall is determined by caspase-4,5 in human cells and caspase-11 in mice. The caspase-4/5/11 then initiates pyroptosis after cleaving GSDMD. Simultaneously, the NLRP3 inflammasome is activated by the amino-

terminal GSDMD-N by caspase-1 processes IL-1β and IL-18 (Ding et al., 2019). Under the stimulation of pathogens, ligand connecting recruits the adaptor protein of the myeloid differentiation protein 88 to the domain of TIR in the region of the cytoplasm of the IL-1β and IL-18 receptor, leading to the autophosphorylation, activation, and recruitment of IL-1R-associated kinase.

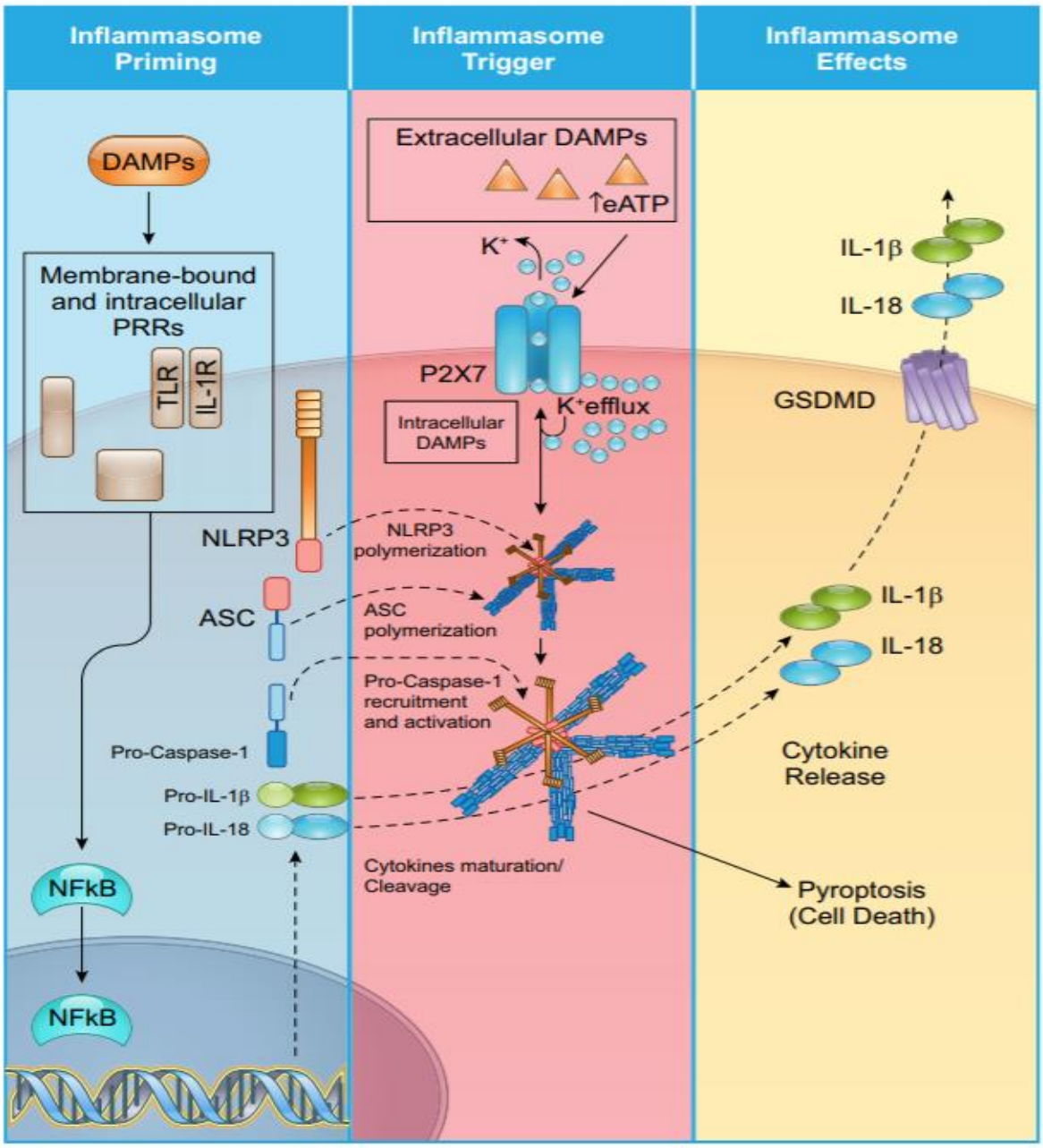

Figure 2: Activation of stages of NOD-like receptors NLRP3.

The receptor then releases the 1R-associated kinase that autoubiquitinates itself, activating TGF β-activated kinase 1 (Dong et al., 2020). The IKB kinase complex is then activated by TAK1 to release NF-KB from IkBa-mediated inhibition. Pyroptosis is then initiated after the NLRP3 inflammasome is activated. Nek7

is an important NLRP3 inflammasome activation component that is grouped as a factor regulating microtubule spindle formation and network nucleation during mitosis (She et al., 2019).

In the system of pyroptosis, Nek7 is involved in the provision or formation of a regular signal functioning upstream of NLRP3. Nek7 as a microtubule dynamics regulator might facilitate the interaction between ASC and NLRP3 (Sun et al., 2020).

Pyroptosis is considered a combination of necrosis and apoptosis involving the loss of the integrity of the plasma membrane and cellular contents release. The domain of GSDMD-N mediates small pores' creation with a diameter of approximately 13nm in the membrane of the plasma. The pores allow the passage of caspase-1 and mature IL-1 β (Murphy & Steenbergen, 2013).

Moreover, the cells often seem to form spherical vesicles and become osmotically swollen around the nucleus. As the cell increases, the nucleus is condensed and spherical, as well as the fragments of DNA. Pyroptosis can be seen by Hoechst 33342/PI double staining, lactate dehydrogenase release, and electron microscope (Mynbaev et al., 2014).

Pyroptosis and Acute Coronary Syndrome (ACS):

Acute Coronary Syndrome is a chronic illness classified by abnormal deposition of lipids in the heart, obstructing the flow of blood, and subsequent rupture of plaque. Both adaptive and innate responses of the immune, involving B lymphocytes, T lymphocytes, neutrophils, macrophages, and monocytes, are important for the progression and initiation of the acute coronary syndrome (Aki et al., 2020). The death of cells can be seen in acute coronary syndrome and is essential in the progression and development of ACS lesions.

Pyroptosis is involved in the progression and creation of ACS by enhancing the release of various inflammatory factors as well as linked to plaque stability (Zhaolin et al., 2019). NLRP3 is the famous inflammasome that closes the gap between inflammation and lipid metabolism due to crystals of cholesterol. Nevertheless, low-density lipoprotein that is oxidized can trigger the inflammasome of NLRP3 to induce pyroptosis (Aki et al., 2020).

Vascular Endothelial Cell Pyroptosis in ACS:

Vascular endothelial cells (VEC) are considered barriers between the vascular wall and blood. The damage of the VEC is the starting point of ACS lesions. Injury of the endothelium is always accompanied by different types of deaths of cells such as pyroptosis (Toshihiko et al., 2020). The integrity of the vascular wall is ruined after pyroptosis leading to local deposition of lipids, instability of plaque, the formation of ACS, and sudden death.

Caspase-1 is expressed abundantly in humans as plaques. Thus, pyroptosis is involved in the hardening of plaque and the formation of ACS (Wang et al., 2018). The inflammasome pathway of caspase-1 can sense inflammatory mediators or elevated lipids, including DAMPs. Proteins related to pyroptosis are then up-regulated, including IL-1 β, caspase-1, and NLRP3, which activates the VEC pyroptosis. Pyroptosis of the vascular endothelial cells promotes the development of ACS, increases vascular permeability, and results in the loss of endothelium integrity (Wang et al., 2020).

Hyperlipidemia induces the production of ROS via an NADPH oxidase-dependent pathway that triggers caspase-1 and NLRP3, leading to pyroptosis of the endothelial cells and inflammation (Li et al., 2021). Early hyperlipidemia enhances the recruitment of monocytes and activation of EC through the caspase-1, protein-1, and sirtuin-1 activator pathway and exacerbates ACS. In the ApoE and caspase-1double knockout mice, monocyte recruitment is inhibited (Li et al., 2021). The action decreases the adhesion molecule expression and the secretion of inflammatory cytokine and cytokines. The outcome provides new insights for the development of target drugs in ACS (Li et al., 2021).

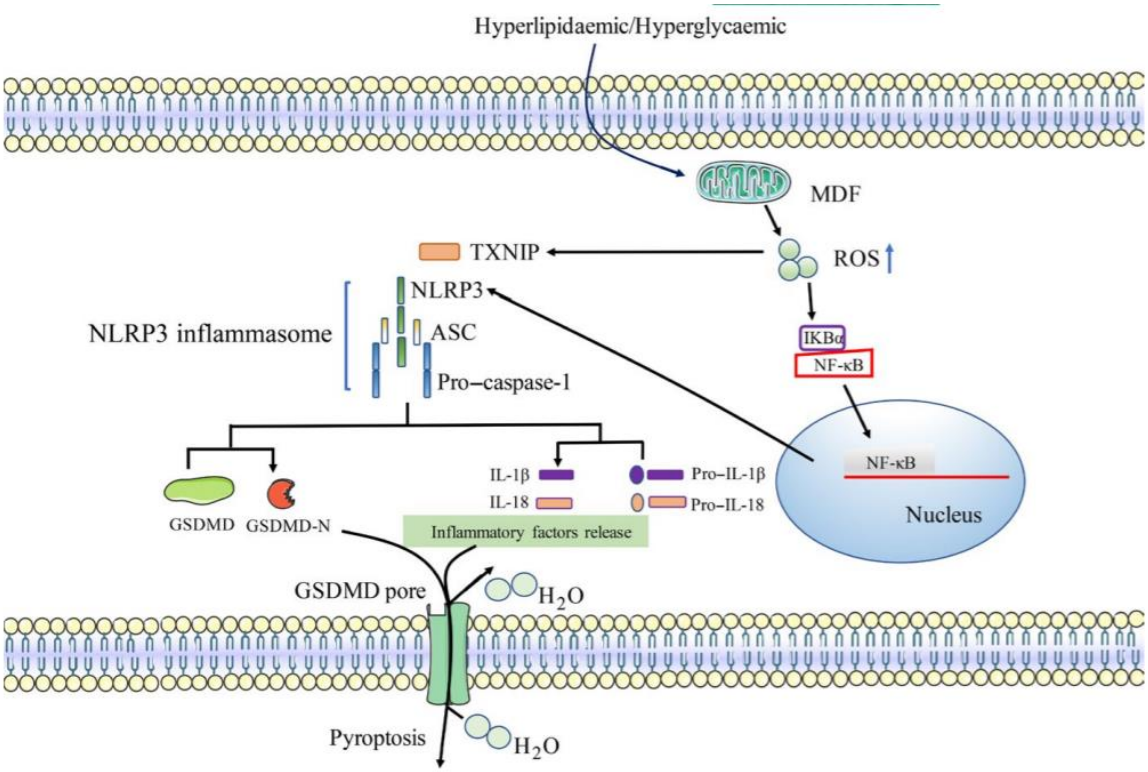

Figure 3: schematic of the primary molecular pathways in pyroptosis in VSMCs and VECs in ACS.

Smoking cigarette is a primary risk factor for ACS and other cardiovascular diseases. Nicotine enhances ACS by inducing vascular endothelial cell pyroptosis. Additionally, nicotine increases the generation of ROS, induces downstream production of IL-1β and IL-18, cleaves pro-caspase-1, and activates the inflammasome of NLRP3, which are all inhibited by the caspase-1 inhibitor (Wang et al., 2014).

In the mouse model of *ApoE*$^{-/-}$, the exposure to nicotine promotes the development of ACS by increasing the pyroptosis of VECs. However, the expression of NLRP3 can decrease the lesion area of ACS and deposition of lipids in the root of the aorta (Noda et al., 2017).

A study by Ouyang et al. (2017) supports the function of mitochondrial ROS generation, mitochondrial calcium fluxes, and mitochondrial adaptors in the activation of inflammasome (Lei et al., 2018). Mitochondrial-derived mtROS is considered as the major source of cellular ROS as well as excessive mtROS is connected with the progression of ACS. The common environmental metal pollutant is Cadmium, which can

cause ACS (Duan et al., 2020). The Cadmium triggers the inflammasome of NLRP3 and the production of downstream caspase-1 and IL-1β by inducing the mitochondrial ROS that mediates VECs pyroptosis thus, promoting the development of ACS (Kuang et al., 2017).

Nevertheless, MicroRNAs are endogenous, and they mediate oxLDL-induced pyroptosis of the vascular endothelial cells. After the down-regulation of TET2, abnormal methylation of DNA takes place, and the dysfunction of the mitochondria induces the production of ROS that activates the inflammasome of NLRP3 (Dziuba et al., 2020). The action results in the activation of caspase-1 that enhances the oligomerization of GSDMD, which activates the formation of pores in the membrane, fragmentation of DNA, and release of mature IL-1β and IL-18 from cells. The action then causes a response of sterile inflammation that contributes to the pyroptotic death of cells, thus promoting ACS (Lin et al., 2020).

Macrophage/Monocyte Pyroptosis in ACS:

The function of adaptive and innate factors of immune in ACS is being valued. The plaques of ACS are categorized by the deposition of lipids in the arterial wall, fiber cap, and infiltration of the immune cells (Mu et al., 2020). In early lesions referred to as fatty streaks, the foaming of macrophages and deposition of lipids can be seen, making complex lesions come out after a certain period, accompanied by necrosis and apoptosis (Pang et al., 2020).

A fibrous cap covers the necrotic core, and the T-cells, mast cells, and macrophages activate its shoulder region, producing pro-inflammatory mediators, which can make plaques unstable. The action can cause fibrous cap rupture resulting in tissue infarction and embolization (Xi et al., 2020). Although macrophages' death in early lesions of ASC is important, a decrease in the number of cells in the plaque can lead to inflammatory response attenuation and reduction in the matrix metalloproteinase synthesis (Liu et al., 2020).

However, macrophages' death in advanced lesions enhances the instability of ACS plaques and the formation of necrotic cores. The death of macrophages in the lesions of ACS causes the release of intercellular lipids, proteases, cytokines, and growth factors to the inflammatory response, thus promoting thrombosis and plaque rupture, causing ACS (Murphy & Steenbergen, 2013).

Low-density cholesterol of lipoprotein and serum total cholesterol is considered CHD risk factors, and oxLDL has a significant effect on ACS. The oxLDL-induced macrophage pyroptosis is essential in plaque stability and the formation of ACS. The crystals of cholesterol, as well as oxLDL in the area of plaque necrosis, activates caspase-1 and NLRP3 for cell pyroptosis induction (Jian et al., 2016). The phenomenon leads to the release of IL-1β and IL-18 macrophages that exacerbate inflammation and ACS. Nevertheless, triglycerides are ACS risk factors that can activate pyroptosis and aggravate the disease (Gan et al., 2020).

IL-1β of the IL-1 family is a crucial cytokine of pro-inflammation primarily produces by activated macrophages/monocytes. On the one hand, neutrophils, macrophages, and monocytes are activated by IL-1β; on the other hand, Th17 and Th1 are induced, thus participating in the initiation of immune and inflammatory responses (Hu et al., 2018). Moreover, IL-1β depletion inhibits ACS and decreases the area of ACS plaque in

IL-1$^{-/-}$ and *Apo E*$^{-/-}$ (Giampieri et al., 2019).

The lack of mtDNA inhibits the development and formation of ACS. RHO0 cells resist apoptosis, and the mtDNA depleted cells resist oxLDL-induced pyroptosis of cells by decreasing ROS production and then inhibiting NLRP3 inflammasome activation. Nonetheless, the mtDNA absence does not affect the oxLDL-induced potential of the mitochondrial membrane and intracellular accumulation of lipids (Zhao et al., 2020). Mitochondria are linked to cardiovascular diseases, and the rupture of the mitochondrial potential of the transmembrane is an indication of the apoptotic cascade.

The depicted disruption takes place before apoptotic features appear in the nucleus. Once the potential of the transmembrane collapses, apoptosis is irreversible (Xu et al., 2018). Additionally, CD36 is a membrane glycoprotein present in different types of cells such as platelets, adipocytes, microvascular ECs, macrophages, and monocytes. Macrophage CD36 takes part in ACS lesions formation by connecting with oxLDL. CD36 is important in the uptake of oxLDL and the formation of cell foam that is the first crucial ACS phase (Yang et al., 2018). CD36 deletion can also inhibit ACS lesions formation.

Platelets CD36 enhances the inflammation of ACS and takes part in thrombus formation after ACS plaques rupture. Nonetheless, CD36 deletion suppresses IL-1β production and targeted CD36 inhibition can decrease the concentration of IL-1β plasma as well as cholesterol crystals deposition in the plaque of ACS, thus inhibiting the progression of ACS (Yang et al., 2018). The human periodontal pathogen of diseases links with the innate receptor of immune, scavenger receptor-B2, and Toll-like receptor-2. TLR2 and SR-B2 promote the activation of NLRP3 inflammasome as well as the production of IL-1β, thus inducing pyroptosis and enhancing the development and formation of ACS (Yu et al., 2019).

Pyroptosis of Vascular Smooth Muscle Cells in ACS:

Vascular smooth muscle cells are important in vascular injury repair through functional and phenotypic transformation. Activated vascular smooth muscle cells have advanced migration and proliferation abilities that promote the repair of the walls of blood vessels (Hacker et al., 2019). However, in ACS chronic inflammation, the function and phenotype of arterial vascular smooth muscle cells become abnormal, leading to differentiation of the VSMCs and increased extracellular formation of the matrix in the plaque region (Guo et al., 2019).

AIM2, one of the HIN-200 family of proteins, is important in inflammasome activation. HFD increases the expression of intercellular cell adhesion molecule-1, GSDMD-N, and AIM2. The overexpression of AIM2 increases the area of plaque lesion as well as pyroptosis of the vascular smooth muscle cells leading to the activation of ACS. AIM2 mediates the activity of GSDMD through the ASC caspase-1 pathway (Gong et al., 2020). Pyroptosis of the VSMCs is also related to the stability of ACS plaques. The reason is that the molecules involved in cell pyroptosis are often expressed in unstable plaque as opposed to stable plaques.

Moreover, in the arterial intima, the extracellular matrix created by the vascular smooth muscle cells such as elastin and collagen are significant fibrous cap components that constitute the ACS plaque (Zeng et al., 2019). The depicted component is linked to the stability of plaque and ACS events. Numerous macrophages

and VSMCs die in the late lesions of ACS. The showcased VSMCs death in ACS might make the fibrous plaque cap unstable and fragile and induce ACS (Yu et al., 2020).

CONCLUSION

Research on different cardiovascular diseases and pyroptosis has rapidly progressed. Many pieces of research have confirmed the significant function of pyroptosis in cardiovascular diseases, precisely, ACS, where different stimuli result in the dysfunction of the mitochondria. In turn, the overproduction of ROS activated NLRP3 inflammasome and increased nuclear NF-Kb translocation that induces pyroptosis to take place in the VSMCs, CMs, and ECs. Moreover, disorienting related molecules in the pyroptotic pathway (IL-1β, caspase-1, AIM2, and NLRP3) affect the progression and occurrence of pyroptosis and ACS, which may provide a potential target for treatment for other cardiovascular diseases.

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