Mitochondria-derived peptides: Promising microproteins in cardiovascular diseases (Review)

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Abstract. Mitochondria-derived peptides (MDPs) are a unique class of peptides encoded by short open reading frames in mitochondrial DNA, including the mitochondrial open reading frame of the 12S ribosomal RNA type-c (MOTS-c). Recent studies suggest that MDPs offer therapeutic benefits in various diseases, including neurodegenerative disorders and types of cancer, due to their ability to increase cellular resilience. Mitochondrial dysfunction is a key factor in the onset and progression of cardiovascular diseases (CVDs), such as atherosclerosis and heart failure, as it disrupts energy metabolism, increases oxidative stress and promotes inflammation. MDPs such as humanin and MOTS-c have emerged as important regulators of mitochondrial health, as they show protective effects against these processes. Recent studies have shown that MDPs can restore mitochondrial function, reduce oxidative damage and alleviate inflammation, thus counteracting the pathological mechanisms that drive CVDs. Therefore, MDPs hold promise as therapeutic agents that are capable of slowing, stopping, or even reversing CVD progression and their use presents a promising strategy for future treatments. However, the clinical application of MDPs remains challenging due to their low bioavailability, poor stability and high synthesis costs. Thus, it is necessary to improve drug delivery systems to enhance the bioavailability of MDPs. Moreover, integrating basic research with clinical trials is essential to bridge the gap between experimental findings and clinical applications.

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Contents

1. Introduction

The central dogma of molecular biology is that genetic information is transferred from deoxyribonucleic acid (DNA) to ribonucleic acid (RNA) and then from RNA to proteins (1). In this process, messenger RNA (mRNA) plays a crucial role as a template for protein synthesis, carrying genetic information that is central to the dogma of molecular biology (2). Of the RNA found in mammalian genomes, ~50% is unable to produce long transcripts because of the absence of large open reading frames; such RNA is known as long noncoding RNA (lncRNA). However, advances in genome sequencing have revealed that, in addition to mRNAs, lncRNAs can also contain short open reading frames (sORFs) that encode fewer than 100 codons. These sORFs within lncRNAs encode functionally stable peptides or microproteins (3-5).

Mitochondria are indispensable for cellular aerobic respiration, facilitating ATP synthesis and sustaining the oxidative respiratory chain (6,7). Beyond their role in energy production, mitochondria regulate redox signaling, cellular senescence, calcium homeostasis, apoptosis and inflammation. Thus, maintaining mitochondrial homeostasis is vital for proper cellular function (7,8). Processes such as pyroptosis, ferroptosis and necroptosis may result from a reduction in mitochondrial numbers. Furthermore, mitochondrial dysfunction can lead to autophagic degradation, loss of membrane potential, excessive reactive oxygen species (ROS) production and structural damage (9). Mitochondrial autophagy impairment has been linked to myocardial fibrosis (10), atherosclerosis (11) and myocardial ischemia/reperfusion injury (12). Additionally, dysregulation of mitochondrial dynamics contributes to the onset and progression of heart failure (13), myocardial infarction (14) and hypertension (15). These findings underscore the critical role of mitochondrial dysfunction in the pathogenesis

of cardiovascular diseases (CADs). Therefore, investigating the mechanisms of mitochondrial involvement in CVDs and exploring potential therapeutic strategies could provide new insights and targets for CVD treatments (16).

Unlike the single genome found in prokaryotic cells, eukaryotic cells possess multiple genomes. The nuclear genome and the mitochondrial genome of eukaryotic cells have coevolved and continuously adapted to each other, ultimately forming a unified dual-genome system within animal cells that stores genetic information and performs various functions (17). The mitochondrial genome contains only 13 protein-coding genes; therefore, ~98% of mitochondrial function-related proteins are encoded by the nuclear genome. Notably, the mitochondrial genome lacks introns and contains only a few noncoding nucleotides between adjacent genes, as well as sORFs that encode functional mitochondria-derived peptides (MDPs). This ability of the mitochondrial genome to encode functionally significant sORFs through short gene sequences not only highlights the complexity of the mitochondrial transcriptome but also provides new avenues for exploring gene expression and functions within the mitochondria (18-22).

Since the discovery of the first mitochondria-derived peptide, humanin (HN), in 2001 (23), eight additional MDPs have been identified, including small HN-like peptides 1-6 (SHLP1-6) (24), the mitochondrial open reading frame of the 12S ribosomal RNA type-c (MOTS-c) (25) and SHMOOSE (26) (Fig. 1). According to the 2024 report by the American Heart Association, CVDs, including coronary heart disease, acute heart failure and stroke, account for ~30% of deaths worldwide, which underscores their significant morbidity and mortality (27). These conditions are characterized by high mortality rates, significant morbidity and increasing prevalence, making them major contributors to population health issues. In recent years, the incidence and mortality rates of CVDs have been increasing among both elderly and younger populations (28-30). MDPs have been shown to have regulatory effects on various CVDs and to have considerable therapeutic potential. The present review discussed the advances in research regarding MDPs in CVDs, aiming to provide new insights for future studies and developments.

HN: The first discovered MDP. In 2001, through functional gene expression screening, Hashimoto *et al* (23) identified a gene capable of encoding a short peptide, which they named HN. HN has been shown to prevent neuronal cell death induced by various familial Alzheimer's disease (AD) genes and amyloid-beta (Aβ) peptides (23); it is encoded by the ORF of 16S rRNA in mitochondrial DNA (mtDNA). In studies related to the nervous system, HN has been shown to inhibit the neurotoxicity induced by Aβ and effectively prevent the onset of AD (31-33). In addition, HN has promise as a treatment for anxiety and for alleviating diazepam-induced memory impairments (34-36).

As a cytoprotective peptide, HN can interact with heat shock protein 90, thereby exerting a protective effect on cardiomyoblasts, dopaminergic neuronal cells and fibroblasts (37). Studies have shown that HN and its analogs can protect retinal pigment epithelium (RPE) cells from oxidative stress by improving mitochondrial function in RPE cells, thereby providing a protective effect on the retina (38,39). Subsequent research revealed that HN regulates mitochondrial function by increasing intracellular ATP levels and respiratory rates, thereby preventing oxidative stress within cells and ultimately providing cytoprotective effects (37-41).

A single injection of HN and its analogs has been shown to improve systemic insulin sensitivity and markedly decrease blood glucose levels in diabetic rats. Therefore, HN is considered to have potential as a therapeutic agent for diabetes (42). In addition, HN can induce the phosphorylation of signal transducer and activator of transcription 3 (STAT3) and extracellular signal-regulated kinase (ERK), thereby reducing cytokine-induced apoptosis in β-cells. These findings suggest that HN has protective effects on pancreatic islets and could aid in the treatment of diabetes (43).

In addition to the aforementioned effects, HN can also participate in the IL-12/IL-27 cytokine network to exert immunoregulatory functions within the testicular environment, suggesting potential therapeutic effects on male infertility and reproductive health (44). Studies have shown that HN is highly expressed in patients with gastric and bladder cancers (45,46), suggesting its potential as a novel therapeutic target for overcoming chemotherapy resistance in cancer treatment (47). HN, as the first identified MDP, has been studied more extensively than other MDPs. However, a number of aspects of HN functions remain unclear, warranting further in-depth exploration.

MOTS-c: A mitochondrial peptide capable of translocating to the cell nucleus. In 2015, through a computational search for potential sORFs in human 12S rRNA, Kim *et al* identified an sORF consisting of 51 base pairs; this sORF can be translated into a 16-amino acid peptide, which they named MOTS-c (48). MOTS-c is a bioactive peptide that can regulate gene expression and cellular metabolism. Although MOTS-c originates from mtDNA, it can translocate from the mitochondria to the nucleus in response to metabolic stress triggers (48). The nuclear translocation of MOTS-c is dependent on 5'-adenosine monophosphate-activated protein kinase (AMPK). Once MOTS-c enters the nucleus, it can bind to nuclear DNA and interact with transcription factors associated with antioxidant response elements (AREs), such as nuclear factor erythroid 2-related factor 2 (Nrf2), thereby regulating gene expression and enhancing cellular resistance to metabolic stress (25,48).

MOTS-c can also increase the levels of 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), an AMPK activator, by inhibiting the folate cycle and *de novo* purine biosynthesis, thereby activating AMPK. The mechanism of action of AICAR involves the phosphorylation-induced inactivation of acetyl-CoA carboxylase, which activates AMPK and stimulates fatty acid oxidation. This action alleviates the allosteric inhibition of carnitine palmitoyl transferase 1 and enhances glucose uptake in muscle cells (21,25,49). MOTS-c targets skeletal muscle, enhancing systemic insulin sensitivity and increasing glucose processing rates by promoting AMPK activation and GLUT4 expression in muscle tissue. This evidence supports the notion that MOTS-c can prevent insulin resistance and may have therapeutic effects against diabetes (25,50).

MOTS-c can increase NAD+ levels and NAD+ serves as an effective activator of sirtuins, playing a crucial role in the aging

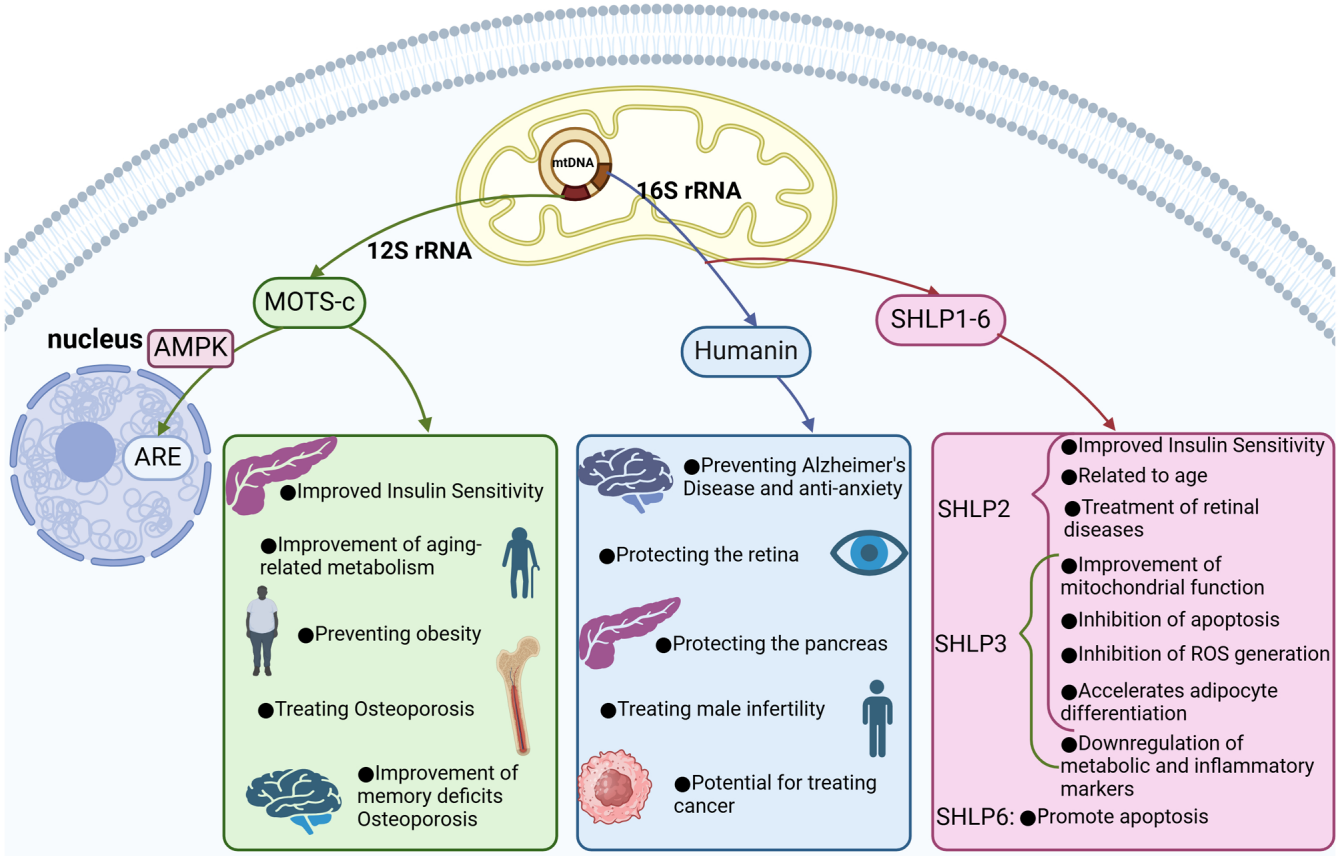

Figure 1. Efficacy of MDPs in treating disease. MOTS-c trans-locates to the nucleus under AMPK activation to regulate transcription factors via AREs, improving systemic insulin sensitivity, preventing obesity and osteoporosis, enhancing memory and promoting metabolic health with age. HN improves memory deficits, protects retinal cells, enhances islet function and addresses male infertility, offering therapeutic potential for AD, diabetes and infertility. SHLP2 improves insulin sensitivity and treats retinal diseases, while SHLP3 supports mitochondrial health, reduces ROS, promotes adipocyte differentiation and downregulates metabolic and inflammatory markers. By contrast, SHLP6 promotes apoptosis. MDPs, mitochondria-derived peptides; MOTS-c, mitochondrial open reading frame of the 12S ribosomal RNA type-c; AMPK, 5'-adenosine monophosphate-activated protein kinase; AREs, antioxidant response elements; HN, humanin; AD, Alzheimer's disease; SHLP, small humanin-like peptides; ROS, reactive oxygen species; mtRNA, mitochondrial RNA.

process (25,51,52). Additionally, MOTS-c levels decline with age, which implies that MOTS-c may play a role in age-related metabolic abnormalities such as reduced insulin sensitivity, impaired fatty acid oxidation, mitochondrial dysfunction, increased obesity, chronic inflammation and decreased metabolic flexibility (25). Treatment with MOTS-c could prevent obesity in mice that were fed a high-fat diet by regulating lipid metabolism and inflammation, as well as by improving mitochondrial function and insulin sensitivity (25). Moreover, MOTS-c treatment has been shown to prevent bone loss by promoting type I collagen production and inhibiting osteoclastogenesis (53), which suggests that MOTS-c acts as a promising therapeutic approach for osteoporosis (54-56). Peripheral treatment with MOTS-c has been shown to enhance the formation and consolidation of objects and spatial recognition memory while also ameliorating Aβ-induced memory deficits. Therefore, MOTS-c is considered a novel potential target for the treatment of cognitive decline in AD (57). As the second MDP discovered after HN, MOTS-c has become a focal point of research in recent years because of its unique association with nuclear DNA. With the continuous advance of MOTS-c research, its multifunctional roles in various diseases have been discovered and confirmed. For example, MOTS-c exerts anti-inflammatory effects by inhibiting the MAPK pathway and stimulating the aryl hydrocarbon receptor (AhR)/STAT3 signaling pathway, which suggests its therapeutic potential for treating sepsis (58). In a murine model of colitis, treatment with MOTS-c increased the levels of phosphorylated AMPK and inhibited the activation of the ERK/JNK pathway, which led to a reduced inflammatory response, increased antiapoptotic capacity and ultimately contributed to the protection against colitis (59). MOTS-c also protects rotenone-treated PC12 neuronal cells from oxidative stress by activating the Nrf2/heme oxygenase 1/NAD(P)H quinone dehydrogenase 1 pathway, which suggests its therapeutic benefits for Parkinson's disease (60). Additionally, MOTS-c improves mitochondrial membrane potential stability by activating the AMPK pathway and reducing inflammation, oxidative stress and cellular damage, thus alleviating cancer-induced bone pain (61). In osteoblasts, MOTS-c promotes the synthesis of type I collagen by activating the TGF-β/SMAD signaling pathway and leading to improvements in osteoporosis (53). With respect to its role in cancer, MOTS-c inhibited the pathological progression of ovarian cancer by attenuating USP7-mediated deubiquitination of LARS1 (62). In addition to its described mechanisms and functions in various diseases, MOTS-c exerts beneficial effects on CADs by regulating inflammation, oxidative stress and apoptosis (63). This aspect is further detailed in the

following section. The multifunctionality of MOTS-c in these diseases underscores its immense potential as a therapeutic target.

SHLP1-6. In 2016, Cobb *et al* (24) conducted a computational search for potential sORFs within 16S rRNA that encode short peptides (20-40 amino acids). They identified six sequences that encode peptides ranging from 20-38 amino acids and named them SHLP1-6. Some of their functions and mechanisms of action have been elucidated (Table I).

SHLP2 is an insulin sensitizer that markedly improves insulin sensitivity by increasing the glucose infusion rate, inhibiting hepatic glucose production and enhancing peripheral glucose uptake (24). As age increases, the level of SHLP2 in the bloodstream decreases, which may be indicative of an association with age-related diseases (24). SHLP2 treatment of senescent RPE cells revealed that SHLP2 can inhibit cell apoptosis, primarily through the downregulation of caspase family gene expression (64). SHLP3 can downregulate the expression of metabolic and inflammatory markers, thereby inhibiting the generation of ROS; additionally, it mediates ERK signaling pathways, promoting adipocyte differentiation and ultimately enhancing mitochondrial function and cell survival (44,65). SHLP2 and SHLP3 can both enhance mitochondrial function, reduce cell apoptosis, decrease the production of ROS and accelerate adipocyte differentiation (24). SHLP2 and SHLP3 can prevent staurosporine-induced damage to the mitochondrial membrane and the activation of caspase-3, thereby exerting protective effects on cells. Additionally, SHLP2 can induce the phosphorylation of STAT3 and ERK, further contributing to its protective effects on cellular health (24,43,66). In experiments assessing the effects of SHLP1-6 on cell viability, SHLP2 and SHLP3 increased cell viability and reduced apoptosis in NIT-1 and 22Rv1 cells; by contrast, SHLP6 markedly increased apoptosis in NIT-1 and 22Rv1 cells, demonstrating effects opposite to those of SHLP2 and SHLP3 (24). In addition to identifying the roles of SHLPs in human diseases, researchers sequenced mtDNA from five rodent species and found highly homologous fragments when the sequences of MOTS-c, SHLP4 and SHLP6 were compared. This discovery has significant research implications for exploring the reasons behind hibernation behaviors in these animals as adaptations to cold climates (67).

Currently, research on the functions of SHLP1-6 has not received widespread attention. However, the protective effects and insulin-sensitizing properties of SHLP2 and SHLP3, which are similar to those of HN, highlight their significant potential for treating certain related diseases. There remains a substantial gap in our understanding of the roles and mechanisms of SHLP1, SHLP4, SHLP5 and SHLP6. Nevertheless, existing studies suggest that these peptides can influence cell function and survival, indicating their potential role in regulating cellular homeostasis (Table I). In summary, the discovery and investigation of SHLP1-6 may provide new insights and approaches for future research on various diseases.

SHMOOSE: A recently discovered MDP. In 2022, via various detection methods, Miller *et al* (26) identified a mitochondrial microprotein named SHMOOSE. SHMOOSE is expressed at higher levels in the brains of patients with AD compared with those of healthy individuals (26). Moreover, following intracerebroventricular administration, SHMOOSE markedly alters the transcriptomic profiles of the hypothalamus and hippocampus, enriching genes associated with enhanced mitochondrial transport. These effects contribute to the pathophysiological processes of AD (26). Miller *et al* (26) discovered that in cerebrospinal fluid samples from non-demented individuals, SHMOOSE levels were markedly positively associated with age and total tau protein. Additionally, they found that higher SHMOOSE levels were associated with reduced fractional anisotropy in the corpus callosum and bilateral corona radiata, as measured by Diffusion Tensor Imaging. Based on these findings, they concluded that SHMOOSE is positively associated with white matter integrity. These findings indicate that SHMOOSE has the potential to serve as a biomarker and has significant research potential in the fields of AD and neurobiology. The discovery of SHMOOSE further demonstrates that the exploration of MDP is ongoing, with a number of unknowns still awaiting elucidation.

2. The effect of different MDPs on CVDs

HN in CVDs

Atherosclerosis. The endothelium is a monolayer of endothelial cells that forms a barrier throughout the entire vascular system, separating the vascular wall from the bloodstream; it plays a crucial role in regulating vascular development and maintaining homeostasis (68,69). The core function of the endothelium is to maintain the balance between vascular dilation and constriction (70,71). When the endothelium is stimulated and damaged, the balance between vascular dilation and constriction is disrupted, ultimately leading to endothelial dysfunction (72). Endothelial dysfunction is considered an early manifestation of atherosclerosis (69,73). In the initial stages of atherosclerosis, a dysfunctional endothelium produces proinflammatory cytokines and decreases the activity of nitric oxide (NO), which has anti-inflammatory properties. This process promotes the recruitment and migration of monocytes from the circulation into the intima, ultimately leading to their differentiation into macrophages (74,75). Macrophages engulf oxidized lipoproteins, forming foam cells, which serve as a hallmark of early atherosclerosis (76). Moreover, when the endothelial layer of arterial blood vessels is damaged, low-density lipoprotein (LDL) permeates the subendothelial space. ROS oxidatively modify LDL, resulting in the formation of oxidized LDL (Ox-LDL). Ox-LDL further increases ROS production, ultimately leading to increased oxidative stress, inflammation and atherosclerotic plaque formation (77-79). In addition to endothelial dysfunction, factors such as inflammation, dyslipidemia, plaque rupture and smoking also contribute to the development of atherosclerosis (80,81).

Studies have shown that serum levels of HN are markedly lower in patients with endothelial dysfunction than in healthy individuals (82,83). This decrease may be associated with coronary endothelial dysfunction, including impaired vascular dilatation, which is typically associated with increased oxidative stress and inflammation. These factors disrupt mitochondrial energy production and structural integrity, leading to mitochondrial dysfunction, which, in turn, contributes to reduced levels of HN (84-86). These findings suggest

Table I. Physiological functions of SHLP1-6.

Mitochondria-derived peptide	Length, amino acids	Sequence	Physiological significance	(Refs.)
SHLP1	24	MCHWAGGASNTGDARGDVFGKQAG		(24)
SHLP2	26	MGVKFFTLSTRFFPSVQRAVPLWTNS	1) Induces the phosphorylation of STAT3 and ERK 2) Blocks staurosporine-induced mitochondrial membrane damage and caspase-3 activation, providing protective effects on cells 3) Functions as an insulin sensitizer 4) Associated with age-related diseases 5) Improves mitochondrial function, reduces apoptosis and ROS production and accelerates adipocyte differentiation 6) Enhances cell viability and decreases apoptosis in NIT-1 and 22Rv1 cells	(24,43,44, 65,66)
SHLP3	38	MLGYNFSSFPCGTISIAPGFNFYRLY FIWVNGLAKVVW	1) Inhibits ROS production, mediates ERK signaling, promotes adipocyte differentiation and blocks apoptosis 2) Induces ERK phosphorylation 3) Exerts protective effects on cells 4) Regulates the expression of metabolic and inflammatory markers 5) Enhances cell viability and reduces apoptosis in NIT-1 and 22Rv1 cells	(24,43,44, 65,66)
SHLP4	26	MLEVMFLVNRRGKICRVPFTFFNLSL	Promotes the proliferation of NIT-1 cells	(24)
SHLP5	24	MYCSEVGFCSEVAPTEIFNAGLVV	Promotes β-cell survival	(24)
SHLP6	20	MLDQDIPMVQPLLKVRLFND	Increases apoptosis in NIT-1 and 22Rv1 cells	(24)

SHLP, small humanin-like peptides.

that serum HN levels could serve as potential indicators for monitoring endothelial dysfunction. ROS can contribute to endothelial dysfunction by reducing the activity of NO in blood vessels and promoting cellular damage (87). Therefore, oxidative stress is considered a crucial mechanism involved in the pathogenesis of endothelial dysfunction and plays a significant role in the occurrence and progression of atherosclerosis (77). Research has shown that HN is expressed in the endothelial cells of both human arteries and veins. Additionally, HN can reduce the levels of ROS and ceramides induced by Ox-LDL in human aortic endothelial cells in a dose-dependent manner, thereby preventing cell apoptosis (88,89). HN exerts its antioxidant effects by inhibiting the production of ROS through the suppression of reduced nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX2) (90). These findings suggest that HN may serve as a potential therapeutic target for atherosclerosis by mitigating oxidative stress.

Colivelin is a hybrid peptide composed of the C-terminus of activity-dependent neurotrophic factor (ADNF) fused with the HN derivative AGA-(C8R) humanin-G (HNG)17 (91). Research has shown that Colivelin can alleviate mitochondrial dysfunction in damaged endothelial cells, playing a critical role in maintaining the structural integrity of the vascular wall (92,93). As Colivelin can help maintain normal vascular structure, it has the potential to serve as a therapeutic agent for treating endothelial dysfunction, thereby exerting beneficial effects on atherosclerosis.

Acute myocardial infarction. Acute myocardial infarction results from the acute blockage of blood flow to the myocardium (94). This sudden blockage can lead to a reduction or complete loss of blood flow to a specific part of the heart, ultimately resulting in cardiac damage or necrosis (95,96). Percutaneous coronary intervention can rapidly and effectively restore blood flow to previously ischemic areas. However, the restoration of blood flow to these ischemic regions results in the production of large amounts of ROS, which can induce cardiomyocyte death and lead to myocardial damage. This phenomenon is referred to as myocardial ischemia-reperfusion injury (96-98).

HNG, in which the 14th amino acid (serine) is replaced by glutamic acid, is a more potent analog of HN. Thummasorn *et al* (97) demonstrated that pretreatment with HNG in a mouse model of myocardial ischemia-reperfusion injury conferred beneficial effects against ischemia-reperfusion damage. Pretreatment with HNG can reduce the generation of ROS, thereby alleviating mitochondrial dysfunction in the heart (97). Additionally, HNG pretreatment decreases the infarct area and the protein expression of B-cell lymphoma-2-associated X protein (Bax), resulting in cardioprotective effects (97). Researchers have shown that *in vitro* treatment with HNG in a mouse model of established ischemia can reduce myocardial infarct size in a dose-dependent manner, resulting in cardioprotective effects (99); after HNG treatment, there was a significant increase in the phosphorylation levels of AMP-activated protein kinase (AMPK) and endothelial nitric oxide synthase (eNOS) in the hearts of the mice, as well as a notable decrease in the levels of Bax and B-cell lymphoma-2 (Bcl-2). These findings suggest that HNG may provide cardioprotection in myocardial reperfusion injury by activating AMPK-eNOS-mediated signaling pathways and regulating apoptotic factors (99). Moreover, administering a high dose of HNG (252 μg/kg) during the ischemic phase increases the level of HN in the damaged myocardium, thereby enhancing the cardioprotective effects of HN against myocardial ischemia-reperfusion injury (94). HNG not only increases the level of HN but also reduces the myocardial infarct size and alleviates cardiac mitochondrial dysfunction (94). The results of the aforementioned studies demonstrate that HN and its derivatives play a beneficial role in the prevention and treatment of ischemia-reperfusion injury. This beneficial effect is attributed primarily to the ability of HN and its derivatives to mitigate mitochondrial dysfunction in damaged myocardial cells, thereby protecting the heart. These findings highlight the potential of HN and its derivatives as novel therapeutic agents for ischemia-reperfusion injury.

Heart failure. The endonuclease G (EndoG) gene has been identified as a pressure-independent determinant of cardiac hypertrophy. Specifically, the absence of EndoG induces cardiomyocyte hypertrophy and increases the production of ROS *in vitro* (100). Cardiomyocyte hypertrophy can be induced through the activation of AKT/ERK phosphorylation and the activation of the mTOR signaling pathway, as well as the inhibition of glycogen synthase kinase-3β (GSK-3β) phosphorylation. These processes collectively induce the transcription of multiple genes, including myocyte enhancer factor 2. During the progression of cardiomyocyte hypertrophy, mitochondrial ROS serve as stimulators that affect these signaling pathways. If such stimulation persists, it may ultimately lead to heart failure (101-104).

The addition of low concentrations of HN to cardiomyocytes from EndoG gene-deficient mice can prevent the accumulation of mitochondrial ROS in these EndoG-deficient cardiomyocytes. Moreover, HN can also inhibit the abnormal growth of EndoG-deficient cardiomyocytes *in vitro*, thereby exerting an antihypertrophic effect on these cells (105). Experiments have demonstrated that HN inhibits cardiomyocyte hypertrophy by restoring ROS levels, cell size and normal proliferation capabilities in EndoG-deficient cells (105). In addition, HN restores the proliferation capacity of EndoG-deficient cells while simultaneously normalizing the phosphorylation ratio of phosphorylated Akt/Akt and the expression of cyclin D (106). This occurs because HN can mitigate the impact of ROS on Akt signaling, thereby restoring Akt phosphorylation and cell proliferation in EndoG-deficient cells; that is, HN can overcome the effects of ROS and restore normal proliferation rates in the absence of EndoG (106). The results from these studies demonstrate that HN can beneficially maintain normal cellular function by reducing intracellular ROS levels, particularly by markedly inhibiting cardiomyocyte hypertrophy. Therefore, HN may exert a beneficial effect on heart failure by suppressing cardiomyocyte hypertrophy, indicating its potential as a therapeutic agent for heart failure.

Myocardial fibrillation. Cardiac fibroblasts are the most abundant stromal cell type in the heart and serve as the primary producers of the extracellular matrix (ECM) within the myocardium. These fibroblasts play crucial roles in maintaining the integrity of the ECM network. The main component of the ECM is collagen, which is deposited by cardiac fibroblasts, provides structural support to cardiac tissue, maintains structural integrity and regulates cell communication and

function (107,108). In response to pathological stimuli such as myocardial infarction, cardiac fibroblasts are activated and differentiate into myofibroblasts, disrupting homeostasis within the cardiac tissue. This process initiates and promotes the occurrence and progression of cardiac fibrosis (109-112). Cardiac fibrosis increases with age and ultimately leads to cardiac dysfunction, which is characterized by the deposition of ECM in the myocardium and the production of myofibroblasts and is often accompanied by diastolic or systolic heart failure. The activation of fibroblasts and their differentiation into myofibroblasts are primarily mediated by TGF-β, which contributes to a profibrotic cardiac microenvironment (113-116).

In a study involving the long-term administration of exogenous HNG to aged mice, HNG increased the percentage of cardiomyocytes in aging hearts, reduced collagen deposition in the cardiac stroma and decreased the proliferation of fibroblasts in the aging myocardium; additionally, HNG downregulated the expression of TGF-β1 and MMP2 in the aging myocardium (117). MMPs are enzymes responsible for the degradation of the ECM and the expression of MMP2 plays a profibrotic role in the heart (113,118). Additionally, HNG can attenuate cardiac fibrosis by activating the Akt/GSK-3β pathway. HNG activates the Akt pathway both *in vitro* and *in vivo*. Activated Akt directly phosphorylates GSK-3β at Ser9, negatively regulating GSK-3β kinase activity, inhibiting the opening of the mitochondrial permeability transition pore and ultimately suppressing myocardial failure and fibrosis (117,119,120). The results from the aforementioned studies indicate that treatment with HN and its derivatives can mitigate myocardial fibrosis in aging hearts (117). Whether HN and its derivatives can serve as potential therapeutic approaches for myocardial fibrosis and related diseases in aging hearts requires further investigation. (Fig. 2).

MOTS-c in CVDs

Myocardial infarction and reperfusion injury. In the treatment of patients with ST-segment elevation myocardial infarction (STEMI), performing percutaneous coronary intervention (PCI) after diagnosis leads to more favorable outcomes than do conventional therapies (121). However, PCI does not always yield positive outcomes. The 'no-reflow phenomenon' is a potential complication associated with PCI (122). The pathogenesis of the no-reflow phenomenon is complex and involves multiple factors, such as atherosclerosis, ischemic injury, myocardial reperfusion injury and coronary microvascular dysfunction, all of which can contribute to its occurrence (123).

A comparison of serum MOTS-c levels in patients with STEMI undergoing PCI with those in healthy individuals revealed that MOTS-c levels are markedly lower in patients with STEMI (124). This decrease is more pronounced as the thrombolysis in myocardial infarction (TIMI) blood flow worsens. Therefore, low MOTS-c levels could be an important predictor of STEMI and reduced MOTS-c levels may play a role in the onset and progression of STEMI (124). Furthermore, studies indicate that as MOTS-c levels decline, TIMI flow worsens, ultimately leading to the no-reflow phenomenon. In patients with STEMI undergoing PCI, MOTS-c levels are markedly elevated, indicating that increased MOTS-c has high sensitivity and specificity for predicting the no-reflow phenomenon. Therefore, MOTS-c is considered a robust and independent predictor of no-reflow occurrence (124). Although an association between MOTS-c levels and STEMI has been established, the underlying physiological mechanisms remain unclear and warrant further investigation.

Vascular calcification. Vascular calcification refers to the abnormal deposition of calcium phosphate crystals in the arterial wall (125); it commonly occurs in vascular lesions associated with diabetes, chronic kidney disease, hypertension and aging, leading to medial sclerosis and the calcification of atherosclerotic plaques (125,126). The prevalence of vascular calcification increases with age (126,127). Vascular calcification typically occurs in both the intima and media layers of the arterial wall. The intimal layer consists of endothelial cells, which are surrounded by a thick layer of elastic fibers. Intimal calcification is associated with dyslipidemia and inflammation, with inflammation contributing to thickening of the intimal layer, ultimately leading to atherosclerosis (128-130). Studies have shown that metformin (131), growth hormone-releasing peptides (132) and death-associated protein kinase 3 (133) can alleviate vascular calcification to varying degrees. These substances achieve this effect by influencing AMPK signaling pathways, which play a key role in reducing vascular calcification. These findings suggest that the AMPK signaling pathway plays a crucial role in regulating the progression of vascular calcification.

In a study in which vascular calcification was induced in rats through treatment with vitamin D3 and nicotine, subsequent treatment with MOTS-c markedly reduced blood pressure, preserved normal heart structure and decreased vascular stiffness (134). These findings demonstrate that MOTS-c can improve cardiovascular and vascular abnormalities caused by vascular calcification (134). Further research revealed that MOTS-c can reverse the downregulation of AMPK expression caused by vascular calcification. Additionally, MOTS-c reduces the levels of angiotensin II type 1 (AT-1) and endothelin B (ET-B), contributing to its protective effects on the cardiovascular system (134). AT-1 and ET-B can participate in the AMPK signaling pathway by binding to their respective receptors (134). A decrease in AT-1 receptor levels plays a crucial role in reducing oxidative stress and preventing the progression of myocardial contractile dysfunction, whereas elevated AT-1 receptor levels can induce myocardial fibrosis and heart failure (135,136). These findings suggest that MOTS-c may alleviate vascular calcification and improve associated cardiac abnormalities by activating AMPK signaling and suppressing the expression of AT-1 and ET-B receptors. Therefore, it is proposed that MOTS-c could serve as a potential anti-calcifying agent for intervention in vascular calcification and may have therapeutic effects in this context.

Atherosclerosis. A study revealed that patients with endothelial dysfunction have markedly lower plasma levels of MOTS-c than patients with normal endothelial function (137). Furthermore, the plasma levels of MOTS-c are positively associated with microvascular and epicardial coronary endothelial function, indicating that lower levels of MOTS-c in plasma are associated with endothelial dysfunction (137). Additionally, preconditioning with MOTS-c in rats or aortic arteries from mice with renal artery stenosis can enhance acetylcholine (ACh)-induced vasodilation, indicating that MOTS-c can improve vascular endothelial function *in vitro* (137).

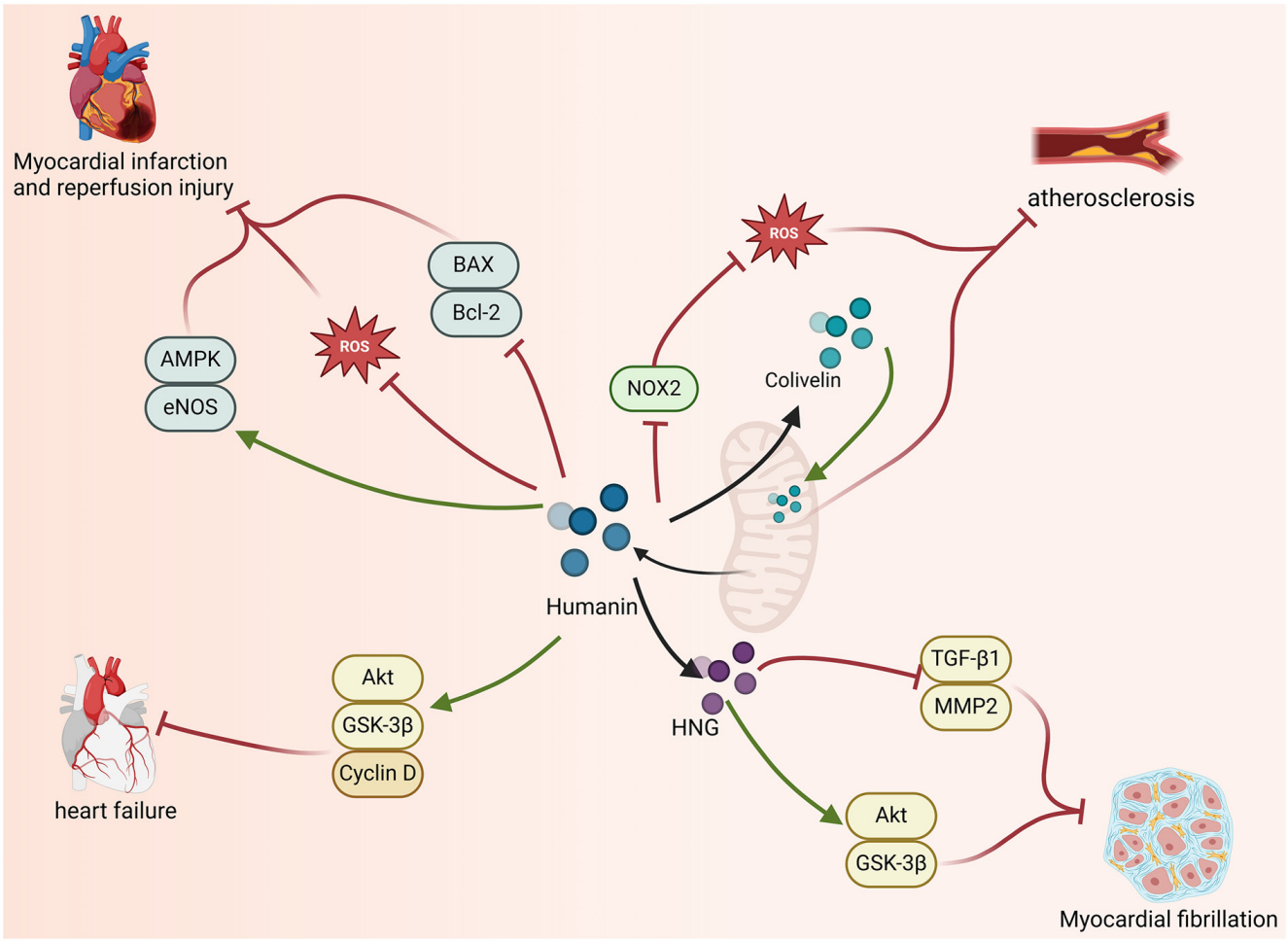

Figure 2. Functions of HN in CADs and related signaling pathways. HN reduces ROS production by inhibiting NOX2, highlighting its therapeutic potential for atherosclerosis. Its derivative, Colivelin, alleviates mitochondrial dysfunction to maintain vascular wall structure and function. HN also inhibits cardiac fibrosis by downregulating TGF-β1 and MMP2 expression while activating the Akt/GSK-3β pathway. The derivative HNG mitigates myocardial infarction and ischemia-reperfusion injury by inhibiting BAX and Bcl-2 expression, reducing ROS production and activating AMPK and eNOS. Additionally, HN benefits heart failure treatment through activation of the Akt/GSK-3β/cyclin D pathway. HN, humanin; CADs, cardiovascular diseases; ROS, reactive oxygen species; NOX2, nicotinamide adenine dinucleotide phosphate oxidase 2; TGF-β1, transforming growth factor-β1; MMP2, matrix metalloproteinase 2; Akt, v-akt murine thymoma viral oncogene homolog; GSK-3β, glycogen synthase kinase-3β; BAX, B-cell lymphoma-2-associated X protein; Bcl-2, B-cell lymphoma-2; AMPK, 5'-adenosine monophosphate-activated protein kinase; eNOS, endothelial nitric oxide synthase; HNG, humanin-G.

In the initial stage of atherosclerosis, a dysfunctional endothelium produces proinflammatory factors and reduces the activity of NO, which has anti-inflammatory properties. This leads to the recruitment and transport of circulating monocytes to the intima, resulting in their differentiation into macrophages (74,75). Research has demonstrated that MOTS-c can inhibit the expression of proinflammatory cytokines, such as TNF-α, IL-6 and IL-1β, while simultaneously increasing the levels of the anti-inflammatory cytokine IL-10 (58). Additionally, MOTS-c exerts its anti-inflammatory effects by inhibiting the phosphorylation of mitogen-activated protein kinase (MAPK)-related proinflammatory pathways and activating AhR-associated anti-inflammatory pathways (58). Furthermore, MOTS-c can inhibit oxidative stress and inflammatory states induced by H_2O_2 in H2c2 cells by activating the Nrf2/ARE pathway and suppressing the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway (138). Additionally, MOTS-c alleviates endothelial dysfunction by inhibiting the MAPK/NF-κB pathway in activated B cells (139).

In summary, MOTS-c can improve vascular endothelial function through anti-inflammatory and antioxidative stress pathways, thereby inhibiting the occurrence and progression of endothelial dysfunction. Given the critical role of endothelial dysfunction in the development of atherosclerosis, MOTS-c is hypothesized to exert therapeutic effects on atherosclerosis by ameliorating endothelial dysfunction (77-79). The effect of MOTS-c on atherosclerosis primarily involves addressing inflammation and endothelial dysfunction; however, the underlying mechanisms warrant further investigation. Additionally, it remains unclear whether MOTS-c has similar therapeutic potential against atherosclerosis induced by other factors, which requires further exploration.

Heart failure. Heart failure is a syndrome that occurs when the heart is unable to pump sufficiently to meet the energy demands of the body (140). The pathogenesis of heart failure includes inflammation, increased oxidative stress, abnormalities in energy metabolism, cardiac cell apoptosis, mitochondrial dysfunction and interstitial fibrosis (140). In a study where heart failure was induced in mice through transverse aortic

constriction surgery, the administration of MOTS-c markedly attenuated the progression of cardiac dysfunction and structural deterioration in the mice; additionally, MOTS-c treatment resulted in a notable reduction in inflammatory responses and an increase in antioxidant capacity (141). Given that inflammation and oxidative stress play crucial roles in the development of heart failure, MOTS-c may hold potential for the prevention and treatment of heart failure progression (141).

The protein levels of neuregulin 1β (NRG1-β) in the serum of heart failure patients are markedly lower than those in healthy individuals (142). NRG1 is a membrane-bound vasoactive peptide that belongs to the epidermal growth factor family; it is released through proteolytic cleavage in response to stimuli such as inflammation, ischemia and oxidative stress in various tissue types, including the heart (143). NRG1 influences cardiomyocytes by activating ErbB tyrosine kinase receptors, leading to downstream signaling through the phosphoinositide 3-kinase (PI3K) and MAPK pathways. This activation ultimately inhibits cell apoptosis and promotes cardiomyocyte proliferation (143,144). In mice with heart failure induced by diabetes, the expression levels of NRG1 and ErbB mRNA decreased; however, following treatment with MOTS-c, the levels of NRG1 and ErbB increased (145). These studies suggest that MOTS-c may achieve therapeutic effects on heart failure by restoring the NRG1/ErbB signaling pathway. Although research on MOTS-c for treating heart failure is limited, the current findings indicate that MOTS-c may hold therapeutic potential. However, further investigations are needed to elucidate the broader mechanisms and physiological roles of MOTS-c in heart failure treatment.

Septic cardiomyopathy. Sepsis is defined as life-threatening organ dysfunction resulting from a dysregulated host response to infection (146). Septic cardiomyopathy is one of the most severe complications of sepsis, with an incidence of 30-60% among septic patients and a mortality rate of 70-90% (147-149). Its primary features include left ventricular dilation and a reduced ejection fraction (EF), which severely impair cardiac function, leading to circulatory failure and multi-organ dysfunction. These complications markedly exacerbate the progression of sepsis (147,148,150,151). Timely intervention in septic cardiomyopathy has the potential to reverse the onset and progression of sepsis, underscoring the critical importance of early detection and targeted therapeutic strategies (152,153). The pathogenesis of septic cardiomyopathy involves dysregulated inflammatory responses, imbalances in calcium homeostasis, mitochondrial dysfunction, oxidative stress and endothelial dysfunction (147,154,155).

Research has shown that in a mouse model of septic cardiomyopathy induced by lipopolysaccharide (LPS), treatment with MOTS-c can reverse the increased transcription levels of inflammatory factors such as IL-1β, IL-4, IL-6 and TNF-α caused by LPS administration. These findings indicate that MOTS-c can effectively decrease the inflammatory response associated with LPS-induced septic cardiomyopathy (155). Additionally, MOTS-c has been shown to reverse the LPS-induced decrease in the expression of the antiapoptotic protein BCL-2 as well as the increase in the levels of the proapoptotic proteins BAX and cleaved caspase-3. Thus, MOTS-c is capable of alleviating cardiomyocyte apoptosis in LPS-induced septic cardiomyopathy (155). In the MOTS-c treatment group, the levels of cellular antioxidants were greater than those in the untreated LPS group; furthermore, MOTS-c was able to eliminate excessive ROS production in cardiomyocytes, indicating that MOTS-c treatment can alleviate the mitochondrial dysfunction and oxidative stress induced by LPS in cardiomyocytes (155). MOTS-c also activates various cardioprotective signaling pathways, including the AMPK, AKT and ERK pathways, while inhibiting multiple proinflammatory signaling pathways, such as the c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38) pathways (155). In summary, MOTS-c may protect normal myocardial function by reducing inflammatory responses in cardiomyocytes, inhibiting cardiomyocyte apoptosis and maintaining mitochondrial homeostasis. Research on the therapeutic effects of MOTS-c in septic cardiomyopathy is still in its early stages. Whether MOTS-c can provide effective clinical treatment for human septic cardiomyopathy and whether it can become a successful therapeutic target are questions that warrant further exploration (Fig. 3).

Other MDPs in CVDs. Hyperlipidemia (HLP) is characterized by lipid metabolism abnormalities and issues related to fat transport, manifesting as elevated cholesterol levels or dysregulated lipoproteins; It is a significant risk factor for conditions such as coronary atherosclerosis, heart failure, hypertension, myocardial infarction and stroke, which are related to CADs (156-159). Increasing evidence suggests that sphingolipids may serve as primary regulators of lipid metabolism (160). A high-fat diet has been shown to increase sphingolipid levels in the liver, adipose tissue and plasma (161). Sphingolipids are synthesized from ceramides through enzymatic transfer mediated by phosphocholine transferase; thus, preventing the *de novo* synthesis of ceramides is crucial for alleviating obesity-related conditions such as hyperlipidemia and atherosclerosis (162,163). The injection of SHLP2 into diet-induced obese mice markedly reduced the plasma levels of CID3126 after treatment (163), with ceramide (CID3126) serving as a precursor for sphingolipid synthesis (163). Concurrently, two glycosylated ceramides, glycosyl-N-palmitoyl-sphingosine and glycosyl-N-steryl-sphingosine, decreased substantially, along with a marked reduction in various types of sphingolipids (163). Additionally, the levels of diacylglycerol, a byproduct generated during sphingolipid synthesis, also tended to decrease in the plasma (163). Collectively, these findings suggest that ceramide synthesis for sphingolipid production is impaired, indicating that SHLP2 may exert beneficial effects on hyperlipidemia by modulating sphingolipid metabolic pathways and altering the concentrations of lipid metabolites in the plasma (163). Therefore, it is proposed that SHLP2 could serve as a potential therapeutic target for conditions associated with hyperlipidemia (Table II).

3. Comparative novelty of the present review

The present review offered an extensive exploration of the roles, mechanisms and therapeutic potential of MDPs in CVDs. Distinct from prior reviews (63,96,164), the present analysis introduced several innovative elements that enhanced the comprehension of the role of MDPs in cardiovascular biology and its therapeutic implications.

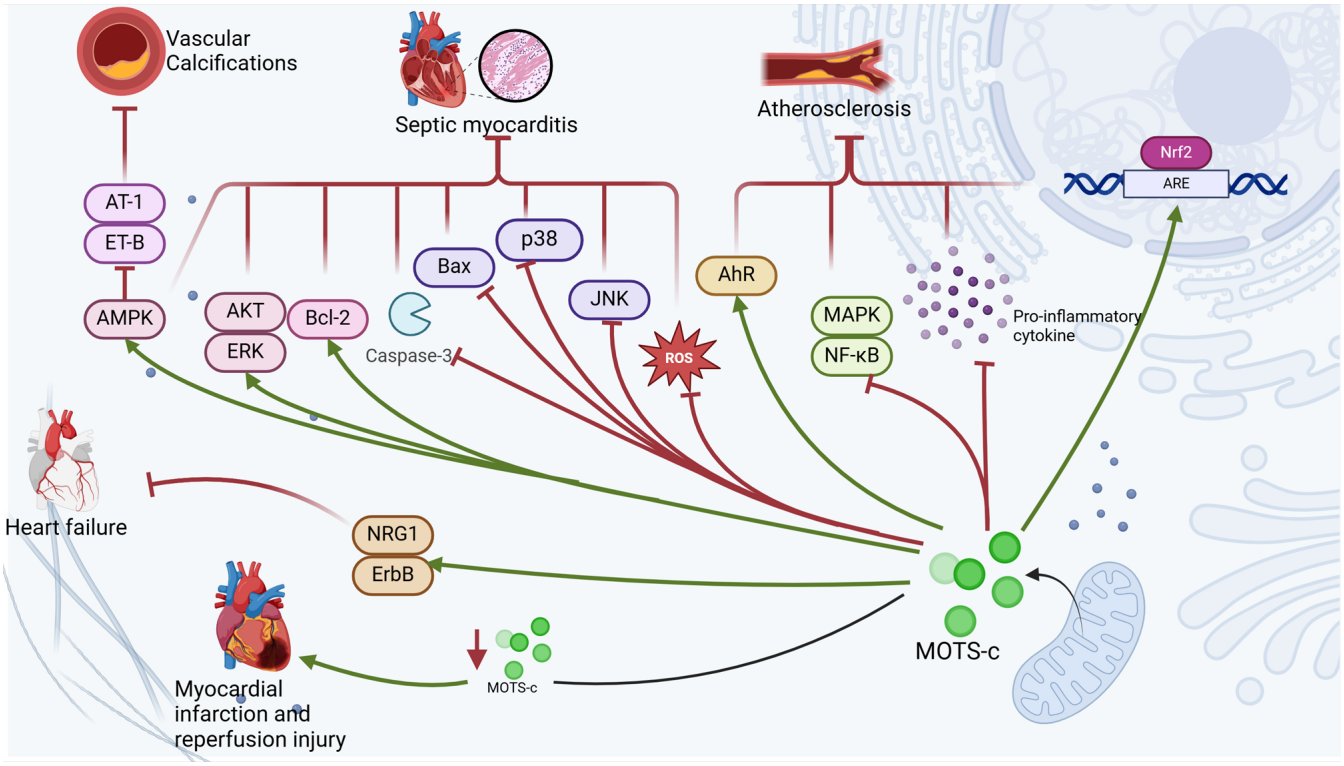

Figure 3. Functions of MOTS-c in CADs and related signaling pathways. MOTS-c activates the AhR and Nrf2/ARE pathways to reduce proinflammatory cytokines and inhibit MAPK/NF-κB signaling, demonstrating potential for atherosclerosis treatment. It also suppresses ROS production, downregulates JNK and p38 signaling, reduces BAX and Caspase-3 expression and promotes Bcl-2 expression while activating the AKT/ERK and AMPK pathways. Additionally, MOTS-c alleviates vascular calcification via the AMPK pathway and reduces AT-1 and ET-B levels. By increasing NRG1/ErbB levels, MOTS-c may aid in heart failure treatment. Reduced serum MOTS-c levels in myocardial infarction and reperfusion injury suggest its potential as a biomarker for cardiac conditions. MOTS-c, mitochondrial open reading frame of the 12S ribosomal RNA type-c; CADs, cardiovascular diseases; AhR, aryl hydrocarbon receptor; Nrf2, nuclear factor erythroid 2-related factor 2; ARE, antioxidant response element; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; ROS, reactive oxygen species; JNK, c-Jun N-terminal kinase; p38, p38 mitogen-activated protein kinase; BAX, B-cell lymphoma-2-associated X protein; Bcl-2, B-cell lymphoma-2; AKT, v-akt murine thymoma viral oncogene homolog; ERK, extracellular signal-regulated kinase; AMPK, 5'-adenosine monophosphate-activated protein kinase; AT-1, angiotensin II type 1; ET-B, endothelin B; NRG1, neuregulin 1; ErbB, a class of receptor tyrosine kinases.

While prior reviews have focused on specific types of MDPs (63,164), this review systematically summarized the roles and mechanisms of various MDPs across different types of CVDs, providing a clearer and more comprehensive overview of the current research landscape. Additionally, the review also pointed out the limitations of MDPs in clinical trials for CVDs, which has often been overlooked in previous works (63,96). Furthermore, the discussion section proposed future research directions, addressing critical gaps in the current literature (164).

In summary, the innovations of this review lie in its comprehensive exploration of the molecular mechanisms of MDPs in CVDs. By systematically summarizing the roles of different MDPs across various types of CVDs and incorporating the most recent research developments, this review offered a thorough and intuitive perspective on the topic. The innovative structural design of this manuscript presented the functions and mechanisms of MDPs in a cohesive manner, enhancing the understanding of their potential therapeutic applications. Additionally, it highlighted the limitations of current research and clinical trials, while proposing future directions to address existing challenges, thereby advancing the scientific knowledge of MDPs in CVDs.

4. Summary and outlook

Currently, the confirmed microproteins identified within the sORFs of mtDNA include HN (23), SHLP1-6 (24), MOTS-c (25) and the recently discovered SHMOOSE (26). Owing to their antioxidant, anti-inflammatory and antiapoptotic properties, MDPs have significant effects on various conditions and diseases, including aging, chronic inflammatory diseases, cancer, neurodegenerative diseases and CVDs (36,47,58,145). The recent discovery of SHMOOSE underlines that our exploration of mitochondrial-derived peptides is just beginning; there may still be a number of unknowns within the mitochondria awaiting discovery, along with numerous questions that remain to be answered.

HN, the first identified MDP, is widely recognized for its therapeutic effects on neurodegenerative diseases (34-36). Research has revealed its significant roles in CADs, diabetes and cancer, particularly in relation to cardiovascular health (43,45,88,94,117). Owing to the antioxidant, antiapoptotic and cytoprotective properties of HN and its analogs, these peptides may have therapeutic potential for cardiovascular conditions such as atherosclerosis (88,90,92), ischemia-reperfusion injury (94,97,99) and myocardial fibrosis (117), suggesting the possibility of developing new

Table II. Roles of MDPs in CVDs.

CVDs	MDPs	Mechanisms of action	Effects on CADs	(Refs.)
Atherosclerosis	HN (overexpression)	Reduces ROS and ceramide levels. Reduces oxidative stress and inhibits apoptosis.	Protects endothelial cells, thereby treating atherosclerosis	(88,90)
	Colivelin (overexpression)	Maintains the structural integrity of the vascular wall.	Improves endothelial functions, thereby treating atherosclerosis	(92)
	MOTS-c (overexpression)	Inhibits proinflammatory cytokines such as TNF-α, IL-6 and IL-1β and stimulates the anti-inflammatory factor IL-10. This is further supported by the suppression of MAPK-related proinflammatory signaling pathways and the activation of AhR and STAT3-related anti-inflammatory pathways, as well as the activation of the Nrf2/ARE pathway and inhibition of the MAPK/NF-κB pathway. Together, these processes mitigate inflammation and oxidative stress, enhance vasodilation and prevent the onset and progression of endothelial dysfunction.	Reduces endothelial dysfunction, thereby treating atherosclerosis	(137,58, 138,139)
Heart failure	HN (overexpression)	Restores normal levels of intracellular ROS, pAkt/Akt ratio and normal expression of cell cycle protein D, which work together to maintain normal function of healthy cells and inhibit cardiomyocyte hypertrophy.	Inhibits cardiomyocyte hypertrophy, thereby treating heart failure	(105, 106)
	MOTS-c (overexpression)	Exerts anti-inflammatory effects, increases cellular antioxidant capacity, inhibits apoptosis, activates the AMPK pathway, restores the NGR1/ErbB pathway and improves cardiac function.	Prevents and treats heart failure	(141, 145)
Myocardial infarction and reperfusion injury	HNG (overexpression)	Reduces ROS production and BAX expression, increases Bcl2 protein expression and activates the AMPK/eNOS pathway, which results in a reduction of the myocardial infarct size, attenuated mitochondrial dysfunction and enhanced myocardial protection.	Reduces the myocardial infarct size and reperfusion injury	(94,97, 99)
Myocardial fibrillation		Activates the Akt/GSK-3β pathway, regulates kinase activity and inhibits the opening of the mitochondrial permeability transition pore.	Inhibits myocardial fibrosis	(117)
Vascular calcification	MOTS-c (overexpression)	Activates AMPK signaling, inhibits AT-1 and ET-B receptor expression, decreases blood pressure, maintains normal cardiac structure and reduces vascular stiffness.	Improves vascular calcification	(134)

Table II. Continued.

CVDs	MDPs	Mechanisms of action	Effects on CADs	(Refs.)
Septic myocarditis	MOTS-c (overexpression)	Reduces mRNA levels of inflammatory cytokines, decreases ROS production, activates cardioprotective signaling pathways, such as the AMPK, AKT and ERK pathways and inhibits pro-inflammatory pathways, such as the JNK, p38 and STAT3 pathways. inhibits inflammation and oxidative stress, reduces cardiomyocyte apoptosis, attenuates mitochondrial dysfunction and protects normal myocardial function.	Treats septic myocarditis	(155)
Hyperlipidemia	SHLP2 (overexpression)	Alters plasma levels of lipid metabolites by modulating the sphingolipid metabolic pathway and preventing ceramide synthesis of sphingomyelin.	Treats hyperlipidemia	(163)

MDPs, mitochondria-derived peptides; CVDs, cardiovascular diseases; CADs, cardiovascular diseases; HN, humanin; ROS, reactive oxygen species; MOTS-c, mitochondrial open reading frame of the 12S ribosomal RNA type-c; MAPK, mitogen-activated protein kinase; AhR, aryl hydrocarbon receptor; STAT3 Nrf2 AREs, antioxidant response elements; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Akt, v-akt murine thymoma viral oncogene homolog; pAkt, phosphorylated Akt; AMPK, 5'-adenosine monophosphate-activated protein kinase; NRG1, neuregulin 1; ErbB, a class of receptor tyrosine kinases; BAX, B-cell lymphoma-2-associated X protein; Bcl-2, B-cell lymphoma-2; eNOS, endothelial nitric oxide synthase; HNG, humanin-G; GSK-3β AT-1, angiotensin II type 1; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; p38, p38 mitogen-activated protein kinase; STAT3, signal transducer and activator of transcription 3.

therapeutic targets for various CADs. While research on HN and its analogs in the context of cardiovascular health is more advanced than research on other MDPs, further investigations are necessary to explore their clinical applications in the future.

MOTS-c is a bioactive peptide that regulates gene expression and cellular metabolism (25,48). Although it originates from mtDNA, it can reach the nucleus in response to metabolic stress, thereby modulating gene expression to cope with such stress (48). The unique properties of MOTS-c have made it a focal point of research in recent years. While studies have established associations between MOTS-c and various cardiovascular-related diseases, including atherosclerosis (58,137-139), vascular calcification (134), heart failure (141,145) and reperfusion injury (124), most studies have focused primarily on observational phenomena rather than elucidating the underlying mechanisms of action. Additionally, the potential relationships of MOTS-c with other CVDs remain to be validated.

Research on SHLP1-6 has focused primarily on SHLP2 and SHLP3, which have been shown to enhance mitochondrial function, reduce apoptosis and ROS production and accelerate adipocyte differentiation (24,43,64). By contrast, SHLP6 has opposite effects (24). The study of SHLP1-6 is still in its early stages and current evidence linking these peptides to CVDs is limited (163). However, the relationship between SHLP2 and hyperlipidemia raises the question of whether SHLP1-6 might also play a role in cardiovascular health, which remains uncertain.

At present, investigations into the role of MDPs in CVDs have focused mainly on HN and MOTS-c, while the effects of other MDPs on cardiovascular health have yet to be thoroughly studied. Regarding HN and MOTS-c, numerous questions remain unanswered. The recent discovery of SHMOOSE prompts the consideration of whether more types of MDPs have yet to be identified. These should be the main issues of concern: First, the molecular mechanisms of MDPs remain incompletely understood, suggesting that their actions may vary across different diseases. Second, most research on MDPs has been conducted in cellular and animal models, with limited clinical data, hindering their application (63,165,166). Third, MDPs exhibit low bioavailability and stability *in vivo* (167,168). Finally, due to technical constraints, the synthesis and detection of MDPs remain costly, presenting both technological and economic challenges to expanding related research (169).

In summary, the associations between MDPs and CVDs are undeniable; however, the underlying mechanisms require further investigation. Thus, it is important to explore the interactions between MDPs and relevant signaling pathways in the regulation of mitochondrial function, inflammation, oxidative stress and apoptosis. Additionally, developing more efficient and stable delivery systems for MDPs could enhance their bioavailability. Furthermore, research on MDPs should focus on bridging the gap between basic studies and clinical trials to verify their safety and therapeutic efficacy in CVD patients. As modern science continues to explore the mitochondrial genome and advances in medical research progress, the therapeutic potential of MDPs for CVD treatment will gradually be revealed.

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Authors' contributions

YR and ZG wrote and drafted the manuscript and figures. LZ, HL and XZ revised the manuscript. XG, XC and HC assisted in manuscript structuring and revisions. MC reviewed and edited the manuscript before submission. Data authentication is not applicable. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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