Role of humanin ; a mitochondrial-derived peptide, in cardiovascular disorders

Luc Rochette, Alexandre Meloux, Marianne Zeller, Yves Cottin, Catherine Vergely

Role of humanin: a mitochondria-derived peptide, in cardiovascular disorders

Rôle de l'humanine : une protéine mitochondriale en pathologie cardiovasculaire

Luc Rochette[1*], Alexandre Meloux[1], Marianne Zeller[1], Yves Cottin[1,2], Catherine Vergely[1]

[1]Equipe d'Accueil (EA 7460) : Physiopathologie et Epidémiologie Cérébro-Cardiovasculaires (PEC2), Université de Bourgogne - Franche Comté, Faculté des Sciences de Santé, 7 Bd Jeanne d'Arc, 21000 Dijon, France

[2]Department of cardiology, CHU Dijon Bourgogne, France

***Corresponding author: E-mail address: luc.rochette@u-bourgogne.fr; Tel: +33-380393291**

Summary The mitochondria produce some specific peptides: mitochondrial derived peptides (MDPs) that mediate transcriptional stress response by its translocation into the nucleus and interaction with DNA. MDPs are regulators of metabolism. This class of peptides comprises: humanin (HN), mitochondrial open reading frame of the 12S rRNA-c (MOTS-c), and Small HN-like peptides (SHLPs). HN inhibits mitochondrial complex 1 activity and limits oxidative stress level in the cell. Data show that MDPs had a role in improving metabolic diseases such as type 2 diabetes (T2D). Moreover, HN could be used as a marker for mitochondrial function in cardiovascular disease or as a pharmacological strategy in patients with endothelial dysfunction. The goal of this review is to discuss the newly emerging functions of HN and its biological role in cardiovascular disorder.

Résumé La mitochondrie produit des peptides spécifiques : les mitochondrial derived peptides (MDPs)qui participent à la réponse transcriptionnelle au stress par translocation dans le noyau et interaction avec l'ADN. MDPs sont des régulateurs du métabolisme. Cette classe de peptides comprend : l'humanine

(HN), les mitochondrial open reading frame of the 12S rRNA-c (MOTS-c), et les Small HN-like peptides (SHLPs). HN inhibe le complexe 1 mitochondrial et limite le niveau du stress oxydatif cellulaire. Des résultats montrent que les MDPs améliorent les désordres métaboliques tels que les diabètes de type 2. Par ailleurs, HN peut être utilisée comme un marqueur de la fonction mitochondriale lors des atteintes cardiovasculaires ou comme une stratégie pharmacologique chez des patients présentant une dysfonction endothéliale. L'objectif de cette revue est d'aborder les fonctions de l'HN récemment définies et son rôle biologique en pathologie cardiovasculaire.

KEYWORDS Mitochondrial-derived-peptides Humanin Cardiovascula Inflammation Marker

MOTS CLES Peptides mitochondriaux Humanine Cardiovasculaire Inflammation Marqueur

Abbreviations: AD, Alzheimer's disease; ATP, adenosine triphosphate ; BAX, BCL-2-associated X apoptosis regulator; BCL2, B-cell lymphoma 2 ; CKD, chronic kidney disease; CNTFR, ciliary neurotrophic factor receptor; DNA, deoxyribonucleic acid; ER, endoplasmic reticulum; ERK1/2, extracellular signal-regulated kinase 1/2; FPRL1, formyl-peptide receptor-like-1 ; GH, growth hormone ; GSH, glutathione ; HF, heart failure ; HN, humanin; HNG, humanin analogue; IGF, insulin-like growth factor-I ; IGFBPs, insulin-like growth factor-binding protein ; I/R, ischemia/reperfusion ; MDP, mitochondrial derived peptides ; MOTS-c, mitochondrial open reading frame of the 12S rRNA-c; mtDNA, mitochondrial DNA ; ORF, open reading frame ; ROS, reactive oxygen species ; RNS, reactive nitrogen species ; SHLPs, small HN-like peptides; T2D, type 2 diabetes ; VCAM, vascular cell adhesion molecule 1.

Introduction

In addition to its role in energy production and metabolism, mitochondria play a key role in apoptosis, oxidative stress, and calcium homeostasis. Dysfunction of mitochondria has been associated with ageing and age-related diseases indicating the importance of this organelle in the maintenance of cell homeostasis. The mitochondria produce some specific peptides that mediate transcriptional stress response by the translocation into the nucleus and interaction with DNA. Mitochondrial derived peptides (MDPs) are regulators of metabolism and various studies have shown that MDPs exerted cytoprotective effects through anti-oxidative stress, anti-inflammatory responses and anti-apoptosis [1, 2].

Mitochondria booked a portion of the original bacterial genomes that co-evolved with nuclear genome. Thus, the mitochondrial and the nuclear genomes had coevolved to coordinate cellular functions, including metabolic and stress response. The mitochondrial genome presents bacterial-like characters: the DNA molecules (mtDNA) are circular, double stranded, with 16,569 nucleotides in humans [3]. The mtDNA encodes functional MDPs. This class of peptides comprises humanin (HN), mitochondrial open reading frame of the 12S rRNA-c (MOTS-c) and Small HN-like peptides (SHLPs) expanding the expression of mitochondrial proteome [4].

Mitochondria can export peptides and also import cytosolic peptides. Mitochondrial protein homeostasis is maintained through proper folding and assembly of newly translated polypeptides. Several factors challenge the mitochondrial protein-folding environment including reactive oxygen species (ROS) that are generated within mitochondria. A major concept in mitochondrial function is the anterograde and retrograde signaling pathways between the mitochondria and the nucleus. In mammalian cells, altered nuclear expression in response to mitochondrial dysfunction is reported; a number of signaling pathways being implicated in this retrograde communication [5].

The mitochondrial unfolded protein response (UPRmt) is also a central part of the mitochondrial response. UPRmt is a mitochondria-to-nuclear communication mechanism that promotes adaptive regulation of nuclear genes

related to mitochondrial response, and is implicated in the cellular homeostasis [6]. UPRs target process depends on the severity of endoplasmic reticulum (ER) stress. When the ER perceives the imbalance in ER homeostasis, the UPR signaling mediates cell survival and adaptation to reduce ER stress [7].

Mitochondrial UPRmt is a mechanism that responds to mitochondrial and cellular stresses. Other than nuclear encoded proteins, mtDNA-encoded peptides, such as humanin and its derivatives are known to have important cellular functions (Table 1) [1].

Mitochondrial genome – an overview

While most mitochondrial proteins are encoded in the nuclear genome, mitochondria have retained a portion of their own genome, the mtDNA. The mitochondrial genome has been compacted to a double stranded circular molecule 11–28 kilobases with few intergenomic region. There is a large non-coding region of that contains regulatory elements for the initiation and termination of transcription of both strands. In human cells, mtDNA consists of 16,569 base pairs, and encodes 37 genes, including 13 polypeptides, two ribosomal RNAs, and 22 tRNAs [8, 9]. mtDNA is inherited from the maternal line, and paternal mtDNA is degraded during fertilization. Mitochondria continuously undergo fusion and fission, which are essential for cell metabolic activities, as well as mtDNA distribution in mitochondria. Alterations in the mtDNA genome have been linked with altered susceptibility to a wide range of age associated diseases. Many human diseases are mechanistically linked to mitochondrial dysfunction and alteration of the the integrity of mtDNA [10]. Mitochondrial noncoding RNA (ncRNAs) in the mitochondrial compartment participate in controlling cardiovascular pathogenesis by regulating mitochondrial energy status, and the expression of genes involved in mitochondrial metabolism [11] Possessing an independent genome and a unique genetic code, mitochondria biosynthesize protective stress response peptides: MDPs.

Humanin (HN) among the Mitochondrial-Derived Peptides (MDPs)

This class of peptides includes, as previously recalled, HN, MOTS-c, and SHLPs. The first MDP discovered back in 2001 was HN; the term based on the potential of this peptide for restoring the "humanity" of Alzheimer's disease (AD) patients. HN was discovered during a search for survival factors in unaffected areas of an AD patient's brain. The initial studies were first performed in cell culture and then followed by *in vivo* studies using both pharmacological mimetics of AD as well as mutant gene: amyloid-β precursor protein. HN is the first small peptide of its kind representing a putative set of MDPs, a novel concept that modifies the established notion about retrograde mitochondrial signaling as well as mitochondrial gene expression. First discovered as a neuroprotective polypeptide; recent studies have shown the beneficial properties of HN in age-related disorders.

HN is a small, secreted, 24 or 21 amino acid-derived peptide depending on cytoplasmic and mitochondrial translation. If HN is translated within the mitochondria, the peptide will be 21 amino acids; and if it is translated in the cytoplasm, then the result is a 24 amino acid peptide [12]. HN is encoded by an HN open reading frame (ORF) within the gene for the 16S ribosomal subunit within the mitochondrial genome [13]. In molecular genetics, an ORF is the part of a reading frame that has the ability to be translated. An ORF is a continuous stretch of codons that begins with a start codon (usually AUG) and ends at a stop codon (usually UAA, UAG or UGA). The evidence suggests that ORFs form a substantial part of our genomes, and that their encoded peptides could have a variety of important cellular functions. Eight distinct MDPs have been currently described whereas thousands of nuclear- encoded ORFs that produce bioactive peptides exist [14].

The human mtDNA was a compact genome that only encoded 13 essential components of the electron transport chain and 24 structural RNAs required for their translation. The mitochondrial genome presents an economy of organization, with no introns and very few noncoding nucleotides between coding sequences [15]. Thirteen nuclear-encoded HN isoforms were demonstrated: HN-like open reading frames were named MTRNR2L1 to MTRNR2L13 after the original HN MTRNR2 gene in the mitochondrial genome. MTRNR2L1–MTRNR2L10 were expressed in most human tissues; MTRNR2 expression being higher compared to the other isoforms. MTRNR2, MTRNR2L1,

MTRNR2L8 and MTRNR2L9 were highly expressed in the heart [13]. It is possible that these isoform genes contribute to the development and physiopathology of cardiac disorders. Few studies have examined the mechanism or conditions by which HN isoforms expression is modified [16].

As HN is a relatively short peptide, exhaustive mutational analysis of the importance of each amino acid has been possible. Molecular manipulations of HN at key amino acids have been shown to offer significant increases in its chemical characteristics. Interestingly, single amino acid substitutions of HN can lead to significant alterations in its potency and biologic functions. S14G-HN in which the serine at position 14 is replaced by glycine, is a highly potent analogue of HN (HNG)**.** HNG-F6A, is another HN analogue with Ser14 to glycine and Phe6 to alanine modifications. The amino acid residues Leu9, Leu10, Leu11, Pro19 and Val20 were found to be important for HN biology. Other HN analogues have also been characterized, including HN-C8P, HN-S7A, and HN-L12A (HN antagonist) [17].

The mechanisms of humanin action

Since MDPs are secretory proteins, they interact with diverse extracellular processes in addition to their intracellular functions. MDPs such as HN exert functions through binding to both intracellular molecules and putative cell membrane receptors. HN has been shown to increase extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation through its receptor binding [18]. Upon stimulation, ERK1/2 is phosphorylated and becomes dissociated from its anchoring proteins, allowing the translocation of ERK1/2 to other subcellular compartments. HN acts as a ligand to two different types of receptors; the seven-transmembrane G-protein-coupled receptor formyl-peptide receptor-like-1 (FPRL1), and a trimeric receptor consisting of ciliary neurotrophic factor receptor (CNTFR), the cytokine receptor WSX-1 and the transmembrane glycoprotein gp130 (CNTFR/WSX-1/gp130). In addition to the direct activation of its receptors, HN prevents cellular apoptosis through the activation of intracellular signaling pathways by inhibiting the translocation of pro-apoptotic proteins to the mitochondria [19, 20].

The first HN receptor: FPRL1 has been linked to neurological disease such as AD. HN acts as an agonist for FPRL1 by inducing Ca^{2+} mobilization and

activation of ERK1/2 signaling. The pathway of G-protein coupled receptors participates to the cytoprotective properties of HN [19]. The trimeric CNTFR/WSX-1/gp130 complex is associated with activation of the gp130-STAT3 axis that is essential for HN activity. Then, HN induces STAT3 activation, which was required for its neuroprotective effects [20].

HN is regulated by insulin-like growth factor-I (IGF-I) and growth hormone (GH). HN and IGF-I levels simultaneously decrease with age and it has been demonstrated that HN levels are directly down-regulated by IGF-I [21]. The biological activity of IGF-I is controlled by six binding proteins: insulin-like growth factor-binding protein (IGFBPs), preventing the IGFs from interacting with the IGF receptor. Concerning the relationship between MDPs and GH metabolism; it has been suggested that GH inhibits levels via IGF-I. Recent review described the role of nutrition and insulin in determining the regulation and actions of the GH-IGF-axis [22]. IGFBP-3 is present in the hypothalamus and inhibits hepatic insulin action via a hypothalamic mechanism [23].

Over the past years, a great number of studies have been dedicated to elucidate the molecular mechanisms of human aging. Among the mechanisms, mitochondrial dysfunction and ROS production is considered in relationship with soluble factors indicated as “mitokines” [24]. Among these mitokines, a peptide such as HN is produced in response to stress. A relationship between circulating HN and age was demonstrated; age is the primary factor that negatively influences the HN levels. In a study; it was reported that the levels of detectable HN in hypothalamus, skeletal muscle, and cortex was decreased with age in rodents, and, in this study, circulating levels of HN would be decreased with age in humans and mice [25]. In return, this evolution was not confirmed in a recent study [26]. HN levels were evaluated in the plasma of 693 volunteers of different age, from 21 to 113 years. Plasma levels of HN are positively correlated with age in humans, and become maximal in centenarians. These data are consistent with the concept that a mitokine such as HN can be considered as a marker of biological age. HN and its homologs are hypothesized to have antiaging activity based on correlative studies. It has been hypothesized that MDPs might have “senolytic” activity, HN and MOTS-c both exacerbate the senescence-associated-secretory-phenotype in senescent cells

by stimulating the secretion of IL-6, IL-1β, IL-8, IL-10 and tumor necrosis factor α [27].

Role of Humanin on oxidative stress

Various studies have shown that HN induced cytoprotective effects through anti-oxidative stress, anti-inflammatory responses and anti-apoptosis, several studies have tested the relationship between HN activity and oxidative stress. Oxidative stress is known to contribute to a range of harmful intracellular events, including DNA damage within the nucleus. This damage may ultimately cause organ dysfunction, and cell death.

It is commonly accepted that the majority of cellular ROS originates from mitochondrial energy metabolism. It is estimated that ~0.2-2% of O_2 consumed by mitochondria is converted to superoxide. Bioenergetics' function of mitochondria is associated with generation of adenosine triphosphate (ATP) via OXPHOS system. Energy is obtained essentially in the mitochondria with the transfer of protons across the inner membranes that produce ATP. The exactly regulation of the synthesis and degradation of ATP (ATP $\leftrightarrow$ ADP + phosphate) is essential to all form of life. Electrons are transferred from NADH, produced in the citric acid cycle in the mitochondrial matrix, to O_2 by a series of large protein complexes in the inner mitochondrial membrane. The two major sites for electron leakage are complexes I and III. ROS and reactive nitrogen species (RNS) are products of cellular metabolism and are well recognized for their dual role as both deleterious and/or beneficial species.

There is evidence that ROS/RNS play key roles in the pathogenesis of various diseases. Oxidative stress is due to an overproduction of ROS/RNS and/or a deficiency in enzymatic (Superoxide Dismutases: SODs, Catalases, Glutathione peroxidases: GSH) and non-enzymatic antioxidant defenses [28, 29]. Oxidative stress is known to contribute to a range of harmful intracellular events, including DNA damage within the nucleus. This damage may ultimately cause organ dysfunction, and cell death. Enhanced generation of ROS and oxidative stress occurs in mitochondria as a consequence of an overload of glucose and oxidative phosphorylation. ER stress plays an important role in oxidative stress, as it is also a source of ROS. The tight interconnection between mitochondria and ER means that the ROS generated in mitochondria promote ER stress [30, 31]. In the context of the oxidative stress, HN treatment inhibits caspases activities and upregulates GSH; GSH inducing a major protection against ROS and RNS [32].

Studies demonstrated that HNG inhibited mitochondrial complex 1 activity and prevented mitochondrial dysfunction and oxidative stress induced by H_2O_2 in isolated cardiac mitochondria [33]. The properties of HN on oxidative stress were supported by findings in cellular and rodent models: HN inhibiting oxidative stress, rescues mitochondrial function, and lowers apoptotic rate. It has been shown that HNG enhances intracellular anti-oxidant capacity, preserves mitochondrial membrane potential, ATP levels and restores mitochondrial integrity in rat's myoblasts (H9c2 cells) [34].

In this field, recent studies provided mechanistic insights into the anti-apoptotic activity of HN. It has been demonstrated that the mitochondrial apoptosis pathway was regulated mainly by members of the B-cell lymphoma 2 (BCL-2) protein group. BCL-2-associated X apoptosis regulator (BAX) plays a pivotal role in the initiation of mitochondria-mediated apoptosis. HN interacts with the membrane-bound Bax preventing the recruitment of cytosolic Bax and its oligomerization in the membrane [35].

Role of Humanin on inflammation

In relationship with the oxidative stress, a role for MDPs in down-regulation of inflammatory responses has been demonstrated *in vivo* and in cell cultures. Inflammation is associated with over-production of ROS in the cell, which can lead to damage of cellular components, and activation of cell death pathways. Redox imbalance, caused by increased free radical's formation and/or reduced antioxidant defense, plays an important role in the development of various diseases. The redox state of the cell is predominantly dependent on an iron redox couple and is maintained within strict physiological limits [45].

Increased oxidative stress, one of the major factors contributing to cell death, plays an important role in the inflammatory process of atherosclerosis. In this field, a study showed that in a cell culture model using human amniotic epithelial cells, pre-treatment with HN attenuated Ox-LDL-induced ROS formation and apoptosis by 50% [36]. Recently, a study investigated HN and MOTS-c protein expression in skeletal muscle and serum levels in advanced chronic kidney disease (CKD) patients and age-matched controls with normal renal function. Circulating levels of HN were increased in CKD and correlated

positively to circulating inflammatory mediator such as tumor necrosis factor-alpha levels [37].

Experimental studies suggest that HN may have therapeutic potential for the treatment of hyperglycemia associated endothelial dysfunction according to their anti-inflammatory properties. HN treatment inhibited high glucose-induced secretion of TNF-α and IL-1β. The reduction of the expression of these proinflammatory cytokines is associated with a reduction of the expression of vascular cell adhesion molecule 1 (VCAM-1) and E-selectin [38].

Role of Humanin in type 2 diabetes (T2D)

Mitochondrial dysfunction and oxidative stress are implicated in the pathogenesis of diabetes. Data provide evidence that mitochondrial dysfunction contributes to glycemic dysregulation and metabolic defects in T2D [39]. The exact mechanisms underlying the disease are unknown; however, there is growing evidence that excess generation of ROS, largely due to hyperglycemia, causes oxidative stress in a variety of tissues. In T2D patients, oxidative stress is closely associated with chronic inflammation [40]. Data suggested that MDPs had a role in improving T2D [41]. The aim of recent studies was to determine MDP levels in normal, prediabetes and diabetes subjects. HN levels are decreased in T2D and correlate with HbA1c. It is possible that marked increase in ROS levels may act to recruit HN from several tissues in the body to damaged areas. In these conditions, HN exerts an antioxidative stress action; this effect could preserve cell survival. In human aortic endothelial cells, HN has been shown to prevent apoptosis by decreasing the ROS production [34].

It was also shown that HN promotes mitochondrial biogenesis in pancreatic cells, acts like an insulin sensitizer, and alters amino acid plasma concentration as well as lipid metabolism [42]. There is increasing evidence of HN involvement in different pathological conditions. Given its protective properties, HN may represent a novel treatment option to lessen the cellular damage caused by T2D. Altered HN levels in T2D could serve as a potential biomarker [41].

Role of Humanin in cardiovascular pathologies

HN protein is endogenously expressed in the heart with the greatest levels found in cardiomyocytes. The expression of the protein is regulated, with levels increasing in the left ventricle in response to a severe stress [43]. The potential cardioprotective mechanisms of HN have been shown to involve both the extracellular and intracellular signaling pathways. Experimental and clinical results are in support of studies regarding the anti-apoptotic effects of HN on the vasculature and the heart.

Regarding cardiovascular diseases, HN has been shown to be expressed in the endothelial layer of human blood vessels including the mammary artery, atherosclerotic coronary artery, and greater saphenous vein. It has been demonstrated that the level of plasma HN decreased significantly in older healthy individuals compared with the younger healthy volunteers and therefore may be associated with the increased risk of atherosclerotic progression in elderly patients [36]. A lower circulating HN level has been described in patients with coronary endothelial dysfunction compared to healthy controls [44]. It is envisaged that endogenous HN levels act to preserve coronary endothelial function. In the field of the development of atherosclerotic coronary disease; it has been reported that the endogenous HN levels in plaque specimens were higher in symptomatic compared to asymptomatic patients. A possible reason for this discrepancy might be due to the disparities of the vascular and metabolic diseases. In these clinical conditions from patients with coronary artery disease, HN levels found could be a compensatory response to conditions of chronic stress [45].

In experimental studies, it was reported that HN may have a protective effect on endothelial function and progression of atherosclerosis by modulating oxidative stress and apoptosis in the developing plaque. Daily intraperitonial injection of the HN analogue HNGF6A for 16 weeks prevented endothelial dysfunction and decreased atherosclerotic plaque size in the proximal aorta of ApoE-deficient mice fed on a high cholesterol diet, without showing direct vasoactive effects or cholesterol-reducing effects. HN was expressed in the endothelial layer on the aortic plaques. HNGF6A treatment reduced apoptosis and nitrotyrosine immunoreactivity in the aortic plaques without affecting the systemic cytokine profile [46].

Progressive changes to myocardial structure and function occur with aging, often associated with underlying pathologies. It is now well established that the aged heart is characterized by fibrotic remodeling. Furthermore, studies using aged animal models suggest that interstitial remodeling with disease may be age-dependent. Age-associated changes in cardiac physiology occur at the cellular, extracellular and whole-heart levels. Apoptotic or necrotic pathways may be responsible for the progressive loss of myocytes with age. Impaired relaxation and therefore diastolic dysfunction is a common characteristic of the aged heart, and it is evident that myocardial fibrosis may be a contributing factor leading to this phenotype [47].

Based on the cytoprotective effects of HN under oxidative stress it has been hypothesized that exogenous HN treatment may be beneficial to aging hearts. It has been reported that exogenous HNG treatment attenuates myocardial fibrosis and apoptosis in aging mice. This cardioprotective effect of HN is associated with activation of Akt/GSK-3β signaling in relationship with an alteration in pro-apoptotic factors. HN treatment significantly increased the ratio of cardiomyocytes to fibroblasts in aging hearts [48].

In various studies, the cardioprotective effects of HN against myocardial ischemia/reperfusion (I/R) injury via decreasing cardiac mitochondrial dysfunction and reduction of ischemic area were demonstrated. The initiating factor of I/R injury is a burst of ROS superoxide from the mitochondrial respiratory chain upon reperfusion that initiates a cascade of tissue damage. Various strategies have been developed to prevent oxidative stress by either reducing ROS production or increasing ROS scavenging ability [49]. In this field, HN and HNG that is produced in various organs including the heart, it exerts, as we previously reported, cardioprotective effects by decreasing oxidative stress and apoptosis. In a major study (*in vitro* and *in vivo*) were investigate the effects of HN administered on cardiac arrhythmia, myocardial infarct size, cardiac mitochondrial function, left ventricular function, and the causal mechanisms against myocardial I/R injury. The infarct size in the HNG-pretreated group was markedly smaller than the vehicle-treated group, whereas the infarct size in the HNG-treated groups at other time points showed no difference when compared with the vehicle-treated group. The endogenous HN level showed a marked decreased after I/R injury; at the end of

I/R, total HN level (in the HNG-treated groups), applied during ischemia and at the onset of reperfusion was significantly increased when compared with the vehicle group. Concerning the cellular mechanisms, it is demonstrated that HNG pretreatment reduced cardiac mitochondrial dysfunction as indicated by reducing ROS production, and mitochondrial membrane depolarization [50].

As we reported above, it is possible that isoform genes of HN contribute to the development and physiopathology of cardiac disorders. The genetic predisposition to coronary artery disease has received increasing attention. Most research has focused on HN and its function; studies realized on several subjects have revealed differences in expression of HN-like isoform genes. Associations between DNA nucleotide variations in HN-like nuclear isoform genes and coronary heart disease were established. The top variants in the flanking region analysis were in the MTRNR2L2 and MTRNR2L8 gene regions [16]. DNA copy number change in isoform genes has been found in arteries of patients with coronary heart disease and metabolic comorbidities [51]. Limitation of the studies includes that it was not possible to determine in these studies the contribution of obesity; diabetes and other metabolic diseases in the genetic scores.

Summary and future directions

In Conclusion, as mentioned above, coronary heart disease and HF are generally accompanied with a decline in mitochondrial respiration and represent the cause of metabolic abnormalities. In this field, the diagnostic role of HN as serum marker is envisaged; as we reported, endogenous HN being increased in cardiovascular disease [43]. Therefore, HN could potentially be used as a marker for mitochondrial function in cardiovascular disease or as a treatment strategy to stabilize atherosclerosis in patients with endothelial dysfunction. HN may provide potential therapeutic options for prevention or treatment of age-related diseases [44]. HN or its non-IGFBP-3 binding analogues may provide potential therapeutic options for prevention or treatment of age-related diseases.

Acknowledgements

This work was supported by grants from French Ministry of Research, and from the Conseil Régional de Bourgogne-Franche-Comté, Dijon University Hospital Center and Association de Cardiologie de Bourgogne.

Disclosure of interest

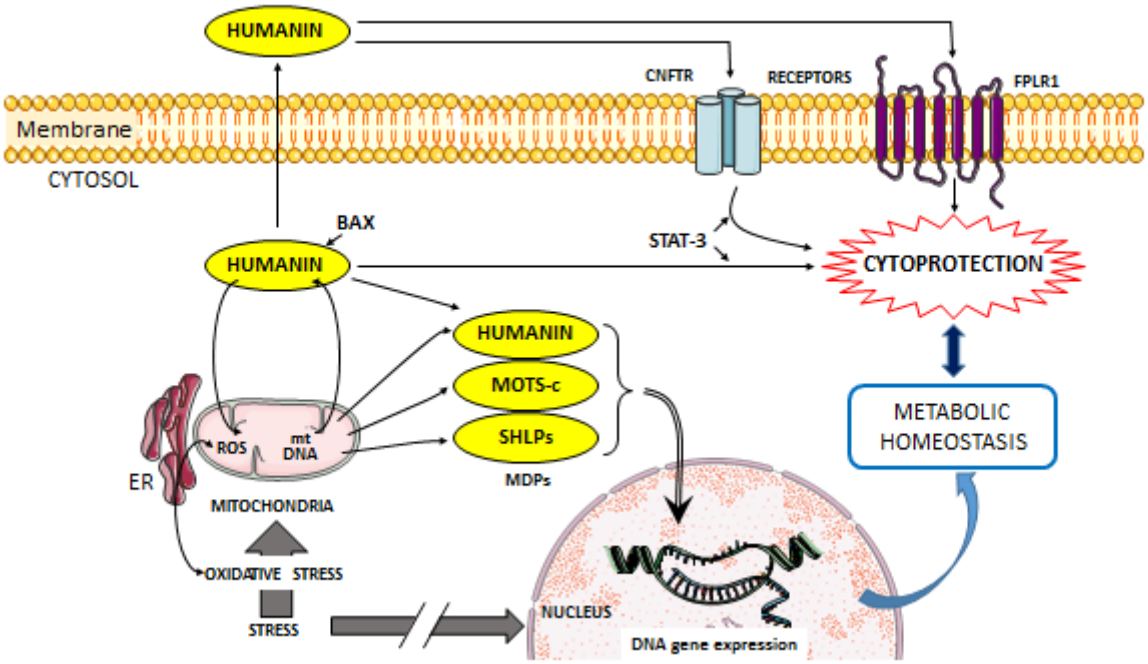
The authors declare that they have no competing interest.

Table 1: Summary of the biological effects of humanin.

CYTOPROTECTION	METABOLIC PROTECTION
- Against injury and oxidative stress - Anti-inflammatory responses - Neuroprotection - Cardioprotection	- Metabolic homeostasis - Increase ATP production - Decrease visceral fat - Glucose-stimulated insulin secretion - Marker of biological age

Figure 1: The protective actions of humanin (HN) through the extracellular and intracellular pathways in the heart

Mitochondrial derived peptides (MDPs) include humanin (HN), MOTS-c (mitochondrial open reading frame of the 12S rRNA-c), and Small HN-like peptides (SHLPs). HN presents both intra-and extra-cellular modes of action. HN activates two types of cell-surface receptors; a trimeric receptor involving CNTFR and relays through the STAT3 signaling pathway. The second HN receptor is FPRL1. HN interacts with the membrane-bound Bax. HN regulates cellular oxidative stress; decreasing mitochondrial reactive oxygen species (ROS). HN production is a response to stress affecting endoplasmic reticulum (ER) and mitochondrial activities.



References

[1] Yong CQY, Tang BL. A Mitochondrial Encoded Messenger at the Nucleus. Cells. 2018;7.
[2] Kim SJ, Xiao J, Wan J, Cohen P, Yen K. Mitochondrially derived peptides as novel regulators of metabolism. J Physiol. 2017;595:6613-21.
[3] Cobb LJ, Lee C, Xiao J, Yen K, Wong RG, Nakamura HK, et al. Naturally occurring mitochondrial-derived peptides are age-dependent regulators of apoptosis, insulin sensitivity, and inflammatory markers. Aging (Albany NY). 2016;8:796-809.
[4] Kim SJ, Mehta HH, Wan J, Kuehnemann C, Chen J, Hu JF, et al. Mitochondrial peptides modulate mitochondrial function during cellular senescence. Aging (Albany NY). 2018;10:1239-56.
[5] Butow RA, Avadhani NG. Mitochondrial signaling: the retrograde response. Mol Cell. 2004;14:1-15.
[6] Yi HS, Chang JY, Shong M. The mitochondrial unfolded protein response and mitohormesis: a perspective on metabolic diseases. J Mol Endocrinol. 2018;61:R91-R105.
[7] Takeda K, Yanagi S. Mitochondrial retrograde signaling to the endoplasmic-reticulum regulates unfolded protein responses. Mol Cell Oncol. 2019;6:e1659078.
[8] Lane N, Martin W. The energetics of genome complexity. Nature. 2010;467:929-34.
[9] Mishra P, Chan DC. Mitochondrial dynamics and inheritance during cell division, development and disease. Nature reviews Molecular cell biology. 2014;15:634-46.
[10] Kondadi AK, Anand R, Reichert AS. Functional Interplay between Cristae Biogenesis, Mitochondrial Dynamics and Mitochondrial DNA Integrity. Int J Mol Sci. 2019;20.
[11] Jusic A, Devaux Y, Action EU-CC. Mitochondrial noncoding RNA-regulatory network in cardiovascular disease. Basic research in cardiology. 2020;115:23.
[12] Yamagishi Y, Hashimoto Y, Niikura T, Nishimoto I. Identification of essential amino acids in Humanin, a neuroprotective factor against Alzheimer's disease-relevant insults. Peptides. 2003;24:585-95.
[13] Bodzioch M, Lapicka-Bodzioch K, Zapala B, Kamysz W, Kiec-Wilk B, Dembinska-Kiec A. Evidence for potential functionality of nuclearly-encoded humanin isoforms. Genomics. 2009;94:247-56.
[14] Pueyo JI, Magny EG, Couso JP. New Peptides Under the s(ORF)ace of the Genome. Trends in biochemical sciences. 2016;41:665-78.
[15] Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, et al. Sequence and organization of the human mitochondrial genome. Nature. 1981;290:457-65.
[16] Eltermaa M, Jakobson M, Utt M, Koks S, Magi R, Starkopf J. Genetic variants in humanin nuclear isoform gene regions show no association with coronary artery disease. BMC Res Notes. 2019;12:759.
[17] Jia Y, Ohanyan A, Lue YH, Swerdloff RS, Liu PY, Cohen P, et al. The effects of humanin and its analogues on male germ cell apoptosis induced by chemotherapeutic drugs. Apoptosis. 2015;20:551-61.
[18] Kim SJ, Guerrero N, Wassef G, Xiao J, Mehta HH, Cohen P, et al. The mitochondrial-derived peptide humanin activates the ERK1/2, AKT, and STAT3 signaling pathways and has age-dependent signaling differences in the hippocampus. Oncotarget. 2016;7:46899-912.
[19] Ying G, Iribarren P, Zhou Y, Gong W, Zhang N, Yu ZX, et al. Humanin, a newly identified neuroprotective factor, uses the G protein-coupled formylpeptide receptor-like-1 as a functional receptor. J Immunol. 2004;172:7078-85.
[20] Hashimoto Y, Kurita M, Aiso S, Nishimoto I, Matsuoka M. Humanin inhibits neuronal cell death by interacting with a cytokine receptor complex or complexes involving CNTF receptor alpha/WSX-1/gp130. Mol Biol Cell. 2009;20:2864-73.

[21] Xiao J, Kim SJ, Cohen P, Yen K. Humanin: Functional Interfaces with IGF-I. Growth Horm IGF Res. 2016;29:21-7.
[22] Clayton PE, Banerjee I, Murray PG, Renehan AG. Growth hormone, the insulin-like growth factor axis, insulin and cancer risk. Nat Rev Endocrinol. 2011;7:11-24.
[23] Muzumdar RH, Ma X, Fishman S, Yang X, Atzmon G, Vuguin P, et al. Central and opposing effects of IGF-I and IGF-binding protein-3 on systemic insulin action. Diabetes. 2006;55:2788-96.
[24] Rose G, Santoro A, Salvioli S. Mitochondria and mitochondria-induced signalling molecules as longevity determinants. Mech Ageing Dev. 2017;165:115-28.
[25] Muzumdar RH, Huffman DM, Atzmon G, Buettner C, Cobb LJ, Fishman S, et al. Humanin: a novel central regulator of peripheral insulin action. PLoS One. 2009;4:e6334.
[26] Conte M, Ostan R, Fabbri C, Santoro A, Guidarelli G, Vitale G, et al. Human Aging and Longevity Are Characterized by High Levels of Mitokines. J Gerontol A Biol Sci Med Sci. 2019;74:600-7.
[27] Mendelsohn AR, Larrick JW. Mitochondrial-Derived Peptides Exacerbate Senescence. Rejuvenation Res. 2018;21:369-73.
[28] Rochette L, Lorin J, Zeller M, Guilland JC, Lorgis L, Cottin Y, et al. Nitric oxide synthase inhibition and oxidative stress in cardiovascular diseases: possible therapeutic targets? Pharmacol Ther. 2013;140:239-57.
[29] Rochette L, Zeller M, Cottin Y, Vergely C. Redox Functions of Heme Oxygenase-1 and Biliverdin Reductase in Diabetes. Trends Endocrinol Metab. 2018;29:74-85.
[30] Burgos-Moron E, Abad-Jimenez Z, Maranon AM, Iannantuoni F, Escribano-Lopez I, Lopez-Domenech S, et al. Relationship Between Oxidative Stress, ER Stress, and Inflammation in Type 2 Diabetes: The Battle Continues. J Clin Med. 2019;8.
[31] Moltedo O, Remondelli P, Amodio G. The Mitochondria-Endoplasmic Reticulum Contacts and Their Critical Role in Aging and Age-Associated Diseases. Front Cell Dev Biol. 2019;7:172.
[32] Rochette L, Vergely C. Coronary artery disease: Can aminothiols be distinguished from reactive oxygen species? Nature reviews Cardiology. 2016;13:128-30.
[33] Thummasorn S, Shinlapawittayatorn K, Khamseekaew J, Jaiwongkam T, Chattipakorn SC, Chattipakorn N. Humanin directly protects cardiac mitochondria against dysfunction initiated by oxidative stress by decreasing complex I activity. Mitochondrion. 2018;38:31-40.
[34] Klein LE, Cui L, Gong Z, Su K, Muzumdar R. A humanin analog decreases oxidative stress and preserves mitochondrial integrity in cardiac myoblasts. Biochem Biophys Res Commun. 2013;440:197-203.
[35] Morris DL, Kastner DW, Johnson S, Strub MP, He Y, Bleck CKE, et al. Humanin induces conformational changes in the apoptosis regulator BAX and sequesters it into fibers, preventing mitochondrial outer-membrane permeabilization. J Biol Chem. 2019.
[36] Bachar AR, Scheffer L, Schroeder AS, Nakamura HK, Cobb LJ, Oh YK, et al. Humanin is expressed in human vascular walls and has a cytoprotective effect against oxidized LDL-induced oxidative stress. Cardiovasc Res. 2010;88:360-6.
[37] Liu C, Gidlund EK, Witasp A, Qureshi AR, Soderberg M, Thorell A, et al. Reduced skeletal muscle expression of mitochondrial-derived peptides humanin and MOTS-C and Nrf2 in chronic kidney disease. Am J Physiol Renal Physiol. 2019;317:F1122-F31.
[38] Wang X, Wu Z, He Y, Zhang H, Tian L, Zheng C, et al. Humanin prevents high glucose-induced monocyte adhesion to endothelial cells by targeting KLF2. Mol Immunol. 2018;101:245-50.
[39] Ramanjaneya M, Bettahi I, Jerobin J, Chandra P, Abi Khalil C, Skarulis M, et al. Mitochondrial-Derived Peptides Are Down Regulated in Diabetes Subjects. Front Endocrinol (Lausanne). 2019;10:331.
[40] Rochette L, Zeller M, Cottin Y, Vergely C. Diabetes, oxidative stress and therapeutic strategies. Biochimica et biophysica acta. 2014;1840:2709-29.
[41] Lee C, Zeng J, Drew BG, Sallam T, Martin-Montalvo A, Wan J, et al. The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance. Cell Metab. 2015;21:443-54.

[42] Qin Q, Jin J, He F, Zheng Y, Li T, Zhang Y, et al. Humanin promotes mitochondrial biogenesis in pancreatic MIN6 beta-cells. Biochem Biophys Res Commun. 2018;497:292-7.
[43] Muzumdar RH, Huffman DM, Calvert JW, Jha S, Weinberg Y, Cui L, et al. Acute humanin therapy attenuates myocardial ischemia and reperfusion injury in mice. Arterioscler Thromb Vasc Biol. 2010;30:1940-8.
[44] Widmer RJ, Flammer AJ, Herrmann J, Rodriguez-Porcel M, Wan J, Cohen P, et al. Circulating humanin levels are associated with preserved coronary endothelial function. Am J Physiol Heart Circ Physiol. 2013;304:H393-7.
[45] Zacharias DG, Kim SG, Massat AE, Bachar AR, Oh YK, Herrmann J, et al. Humanin, a cytoprotective peptide, is expressed in carotid atherosclerotic [corrected] plaques in humans. PLoS One. 2012;7:e31065.
[46] Oh YK, Bachar AR, Zacharias DG, Kim SG, Wan J, Cobb LJ, et al. Humanin preserves endothelial function and prevents atherosclerotic plaque progression in hypercholesterolemic ApoE deficient mice. Atherosclerosis. 2011;219:65-73.
[47] Horn MA, Trafford AW. Aging and the cardiac collagen matrix: Novel mediators of fibrotic remodelling. J Mol Cell Cardiol. 2016;93:175-85.
[48] Qin Q, Mehta H, Yen K, Navarrete G, Brandhorst S, Wan J, et al. Chronic treatment with the mitochondrial peptide humanin prevents age-related myocardial fibrosis in mice. Am J Physiol Heart Circ Physiol. 2018;315:H1127-H36.
[49] Lecour S, Rochette L, Opie L. Free radicals trigger TNF alpha-induced cardioprotection. Cardiovasc Res. 2005;65:239-43.
[50] Thummasorn S, Apaijai N, Kerdphoo S, Shinlapawittayatorn K, Chattipakorn SC, Chattipakorn N. Humanin exerts cardioprotection against cardiac ischemia/reperfusion injury through attenuation of mitochondrial dysfunction. Cardiovasc Ther. 2016;34:404-14.
[51] Nazarenko MS, Sleptcov AA, Lebedev IN, Skryabin NA, Markov AV, Golubenko MV, et al. Genomic structural variations for cardiovascular and metabolic comorbidity. Sci Rep. 2017;7:41268.