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Mitochondrial-derived peptides: Antidiabetic functions and evolutionary perspectives

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Abstract

Mitochondrial-derived peptides (MDPs) are a novel class of bioactive microproteins encoded by short open-reading frames (sORF) in mitochondrial DNA (mtDNA). Currently, three types of MDPs have been identified: Humanin (HN), MOTS-c (Mitochondrial ORF within Twelve S rRNA type-c), and SHLP1–6 (small Humanin-like peptide, 1 to 6). The 12 S ribosomal RNA (*MT-RNR1*) gene harbors the sequence for MOTS-c, whereas HN and SHLP1–6 are encoded by the 16 S ribosomal RNA (*MT-RNR2)* gene. Special genetic codes are used in mtDNA as compared to nuclear DNA: (i) *ATA* and *ATT* are used as start codons in addition to the standard start codon *ATG*; (ii) *AGA* and *AGG* are used as stop codons instead of coding for arginine; (iii) the standard stop codon *UGA* is used to code for tryptophan. While HN, SHLP6, and MOTS-c are encoded by the H (heavy owing to high guanine + thymine base composition)-strand of the mtDNA, SHLP1–5 are encoded by the L (light owing to less guanine + thymine base composition)-strand. MDPs attenuate disease pathology including Type 1 diabetes (T1D), Type 2 diabetes (T2D), gestational diabetes, Alzheimer's disease (AD), cardiovascular diseases, prostate cancer, and macular degeneration. The current review will focus on the MDP regulation of T2D, T1D, and gestational diabetes along with an emphasis on the evolutionary pressures for conservation of the amino acid sequences of MDPs.

Keywords

Mitochondrial peptides; Diabetes; Humanin; MOTS-c; small humanin-like peptides

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CRediT authorship contribution statement

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1. Introduction

Mitochondria, originated from once free-living α-proteobacteria [1-3], are responsible for aerobic generation of ATP: the molecular currency that provides the chemical energy to fuel vital cellular processes [4]. In addition, mitochondria form highly connected networks called mitochondrial reticulum and communicate with the nucleus through retrograde signaling, which allows communication from mitochondria to the nucleus via secondary messengers including Ca^{2+}, ATP/ADP, NAD^+/NADH ratios, and other small molecules [5,6]. Due to their bacterial origin, mitochondrial genome inherited bacterial-like traits including circular and double-stranded DNA molecules (mtDNA), which are small (16,569 nucleotides in humans) and compact. mtDNA contains 37 genes that encode 13 subunits of the oxidative phosphorylation (OXPHOS) system, two ribosomal RNAs (rRNAs), and 22 transfer RNAs (tRNAs) [7]. The mtDNA has no introns but a few non-coding nucleotides between adjacent genes and “small open reading frames” (sORF ≤ 300 nucleotides) that encode functional “mitochondrial-derived peptides” (MDPs, 16–38 amino acids long) [8]. Three types of MDPs have so far been identified: Humanin (HN), MOTS-c (Mitochondrial ORF within Twelve S rRNA type-c) and SHLP (small Humanin-like peptide, 1 to 6), expanding the expression of mitochondrial proteome [9]. MDPs, released into the body via paracrine and endocrine pathways, exert diverse functions as cytoprotective agents, such as maintaining cell viability and mitochondrial function under stress; are involved in cellular metabolism and cell survival and act in response to inflammation and oxidative stress [10-12].

Type 2 diabetes (T2D), the most common type of diabetes in adults (>90%), is characterized by hyperglycemia from to progressive loss of insulin secretion from the β-cells superimposed on a background of insulin resistance (IR), leading to relative insulin deficiency. Obesity (body-mass index [BMI]>30 kg/m^2) is the strongest risk factor for T2D in East Asian populations [13,14] and is associated with metabolic abnormalities resulting in IR [15]. IR is associated with decreases in insulin-mediated glucose uptake in skeletal muscle and white adipose tissue [16-18], and an increase in hepatic glucose production (HGP) [19]. In contrast, type 1 diabetes (T1D) is characterized by autoimmune destruction of pancreatic β-cells, resulting in absolute insulin deficiency and hyperglycemia and is more common in males [20-23]. T1D accounts for ~5% of diabetes in adults. The prevalence of diabetes globally counts to 536.6 million people as of 2021 and is predicted to reach a 783 million by 2045 [24]. T2D causes mitochondrial dysfunction by reducing bioenergetic capacity and increasing production of reactive oxygen species (ROS) [25,26]. In fact, diabetes is associated with premature death, caused mainly by coronary artery disease [27-29], stroke [30-32], or renal dysfunction [33-36]. The levels of MDPs have been reported to decrease in diabetes, which results in metabolic dyshomeostasis [37]. Administration of MDPs to rodents or exposure of primary/immortalized cell lines with MDPs was shown to increase insulin sensitivity, reduce lipid accumulation, promote mitochondrial biogenesis, and increase energy expenditure [11,38-41].

In the current review, we will provide an up-to-date knowledge on regulation of T2D and T1D by MDPs. We will also highlight the evolutionary pressures on conservation of the amino acid sequences of MDPs.

2. Mitochondrial genome and mitochondrial-derived peptides

The mtDNA was discovered in 1963 by electron microscopy and described as a bacterial-like circular DNA [42,43]. The near complete sequence for human mtDNA was reported in 1981 [7], which was minimally revised in 1999 [7]. MtDNA in humans is an 16,569 bp long double-stranded circular molecule, composed of heavy (H) and complementary light (L) strands, which encode 11 mRNAs that give rise to 13 subunits of the oxidative phosphorylation (OXPHOS) system, 22 transfer RNAs (tRNAs) and two ribosomal RNAs (rRNAs) that are essential for mitochondrial translation. The H-strand of the mtDNA encodes HN, SHLP6, and MOTS-c, while the L-strand encodes SHLP1–5 [44]. mtDNA uses *ATA, ATT, and ATG* as start codons, *AGA* and *AGG* (which code for arginine in nuclear DNA) as stop codons, and *UGA* (the standard stop codon in nuclear DNA) to code for tryptophan [45-47]. The mitochondrial genome is highly compact as it contains no introns and little non-coding DNA (a ~1 kb sequence known as the non-coding region NCR (mistakenly referred to as the D-loop) and a distant ~30 nucleotide sequence containing the origin of replication for the L-strand OriL) (Fig. 1). The NCR contains one transcription promoter for each strand (light strand promoter – LSP; heavy strand promoter – HSP) as well as the origin of replication for the H-strand (OriH). Almost all mitochondrial genes, including those that encode 12 subunits of the OXPHOS system (the monocistronic *ND1–3, ND5, Cytb* and *COI-III*, and the biscistronic *ND4/ND4L* and *ATP6/ATP8*), 2 mtRNAs (12 S and 16 S), and 14 mt-tRNAs (F, V, L, I, M, W, D, K, G, R, H, S, L, T), are transcribed from the template of the G-rich H-strand under the control of the HSP. The L-strands serve as a template to produce only 1 mitochondrial messenger RNA (mtRNA) that encodes subunit 6 of NADH dehydrogenase (*ND6*), and 8 mt-tRNAs (Q, A, N, C, Y, S, E, and P).

2.1. MTRNR2 gene and the encoded peptides

Emerging studies indicate that the mtDNA contains sORF, expanding the mitochondrial genetic repertoire [11,12,48-50]. The 16 S ribosomal RNA gene is 1559 nucleotides in length, found within the *MTRNR2* gene and spans mtDNA 1671–3229 bp. Humanin (HN), encoded as a 75-bp polycistronic sORF within the 16 S rRNA, was the first sORF to be identified in 2001 in the mtDNA [10,48,51]. In 2016, six additional peptides in the same region of mtDNA as HN (i.e., 16 S rRNA) were identified and named small HN-like peptides (SHLPs):SHLP1 (2490–2561 bp), SHLP2 (2092–2170 bp), SHLP3 (1707–1821 bp), SHLP4 (2446–2524 bp), SHLP5 (2785–2858 bp), and SHLP6 (2992–3051 bp) [11].

2.2. MTRNR1 gene and the encoded peptide

The 12 S 954 bp rRNA gene (*MTRNR1*) spans from 648–1601 bp and encodes for MOTS-c (Mitochondrial ORF within Twelve S rRNA c: 1343–1393 bp) [12], which was discovered in 2015.

3. Primary sequence, physicochemical properties, and evolutionary pressures in the MDPs

The importance of a peptide is usually determined through loss (knockout or siRNA knockdown of the peptide domain) and gain (supplementation of the peptide to knockout/

knockdown organism/cells) of function studies [52-54]. Although attempts have been made on mitochondrial genome editing by CRISPR [55], knockout of mitochondrial ORFs is yet to be achieved. Therefore, HN was knocked down using siRNA, which reduced its antiapoptotic and neuroprotective effects [51, 56]. The other straightforward test to assess the importance of a peptide relies on the evolutionary pressure on preservation of amino acid sequence, which has recently been applied in MDPs [57].

3.1. Gene symbol and primary sequences of MDPs

Below are the gene symbols and the amino acid sequences of the MDPs: MTRNR2: Humanin (MAPRGFSCLLLLTSEIDLPVKRRA).

MTRNR1: MOTS-c (MRWQEMGYIFYPRKLR).

MTRNR2: Small HN-like peptide 1 (SHLP1: MCHWAGGASNTGDARGDVFGKQAG).

MTRNR2: Small HN-like peptide 2 (SHLP2: MGVKFFTLSTRFFPSVQRAVPLWTNS).

MTRNR2: Small HN-like peptide 3 (SHLP3: MLGYNFSSFPCGTISIAPGFNFYRLYFI-WVNGLAKVVW).

MTRNR2: Small HN-like peptide 4 (SHLP4: MLEVMFLVNRRGKICRVPFTFFNLSL).

MTRNR2: Small HN-like peptide 5 (SHLP5: MYCSEVGFCSEVAPTEIFNAGLVV).

MTRNR2: Small HN-like peptide 6 (SHLP6: MLDQDIPMVQPLLKVRLFND).

3.2. Physicochemical properties of MDPs

The physicochemical properties of MDPS (Humanin, MOTS-C. SHLP1. SHLP2. SHLP3. SHLP4. SHLP5, and SHLP6) are provided in Table 1.

3.3. Evolutionary pressures in the MDPs

3.3.1. Humanin—Several cDNAs sharing sequence homology to HN have been identified in plants, nematodes, and rodents demonstrating that HN is evolutionary conserved [48]. Furthermore, amino acid sequence alignments of HN in primates (n = 252), mammals (n = 148) and vertebrates (n = 359) revealed that HN is conserved across vertebrates with seven residues (A^2, F^6, L^9, L^{10}, L^{18}, R^{22}, and R^{23}) showing codon bias ($f_{syn} \geq 0.5$) [57] (Table 2). In vertebrates, two regions are highly conserved, corresponding to the codons for C^8, L^9 and E^{15}, I^{16}. The authors implied to hypothesize that this conservation might be critical for ribosome function, or could be critical for HN function, or a combination of both [57]. Since methionine has only one codon in the standard DNA code, no f_{syn} value is provided in Tables 2-6. “Ter” stands for termination, or the stop codon. Values of $f_{syn} \geq 0.5$ are presented in bold font.

3.3.2. MOTS-c—Amino acid sequence alignments of MOTS-c in primates (n = 254), mammals (n = 178) and vertebrates (n = 348) revealed that MOTS-c has almost no synonymous codon bias ($f_{syn} \geq 0.5$) [57] (Table 3). MOTS-c, however, contains a highly conserved pentapeptide MGYIF in the middle of the sequence.

3.3.3. SHLP1—Amino acid sequence alignments of SHLP1 in primates (n = 217) showed poor start codon conservation (33%) and almost no synonymous codon bias ($f_{syn} \geq 0.5$) [57].

3.3.4. SHLP2—Amino acid sequence alignments of SHLP2 in primates (n = 219), mammals (n = 174) and vertebrates (n = 369) revealed poor sequence conservation and almost no synonymous codon bias ($f_{syn} \geq 0.5$) [57]. In primates, SHLP2 displayed an $f_{syn} > 0.5$ in only one amino acid (L^{29}). L^{15} and Y^{16} showed f_{syn} values close to 0.5 (Table 4).

3.3.5. SHLP3—Amino acid sequence alignments of SHLP3 in primates (n = 221) showed poor start codon conservation (18%) and almost no synonymous codon bias ($f_{syn} \geq 0.5$) [57].

3.3.6. SHLP4—Amino acid sequence alignments of SHLP4 in primates (n = 215), mammals (n = 144) and vertebrates (n = 339) revealed highly conserved N-terminal region, with many invariant bases (L^2, R^{11}, G^{12}, and L^{26} in primates; L^2, V^4, R^{11}, and F^{21} in mammals; R^{11}, G^{12}, and F^{19} in vertebrates), and is flanked by amino acids, L^2 and R^{11} [57] (Table 5). Based on the above sequence conservation, the authors suggested the occurrence of purifying selection in mammals [57].

3.3.7. SHLP5—Amino acid sequence alignments of SHLP5 in primates (n = 216) showed poor start codon conservation (30%) and almost no synonymous codon bias ($f_{syn} \geq 0.5$) [57].

3.3.8. SHLP6—Amino acid sequence alignments of SHLP6 in primates (n = 242), mammals (n = 147) and vertebrates (n = 348) revealed SHLP6 as the most conserved MDP. The following residues showed synonymous codon bias (Q^4, D^5, L^{12}, and V^{15} in primates; Q^4, D^5, and Q^{10} in mammals; D^3, Q^4, Q^{10}, and L^{12} in vertebrates) (Table 6). Unlike other MDPs, the stop codon in SHLP6 is also highly conserved [57]. Like SHLP4, the authors opined that SHLP6 has also undergone purifying selection.

4. Functions of mitochondrial-derived peptides

4.1. HN

The term Humanin (HN) was coined after ‘humanity’ by Hashimoto [48]. HN has a positively charged N-terminal (Met^1-Ala^2-Pro^3-Arg^4), central hydrophobic region (Gly^5-Phe^6-Ser^7-Cys^8-Leu^9-Leu^{10}-Leu^{11}-Leu^{12}-Thr^{13}-Ser^{14}-Glu^{15}-Ile^{16}-Asp^{17}-Leu^{18}), and negatively charged C-terminal (Pro^{19}-Val^{20}-Lys^{21}-Arg^{22}-Arg^{23}-Ala^{24}) (Fig. 2). The above three domains help HN to bind hydrophobic pockets of proteins to form alpha helix [58]. Arg substitution of HN identified two structures – Leu^9-Leu^{11} and Pro^{19}-Val^{20}, which are essential for the secretion of full-length HN [59]. Leu^{10} plays the most crucial role in this function. Utilizing Ala-scanned HN constructs, Yamagishi et al. identified that Pro^3, Ser^7, Cys^8, Leu^9, Leu^{12}, Thr^{13}, Ser^{14}, and Pro^{19} were essential for the neuroprotective function of HN and that Ser^7 and Leu^9 were essential for self-dimerization of HN, which are critical for its neuroprotective action [59].

4.1.1. HN binding partners, receptors and intracellular signaling—HN binds to many binding partners [60-67], some of which are regarded as receptors for HN. HN signals through binding to both intracellular molecules and putative cell membrane receptors [68-72]. Intracellularly, HN binds to B-cell lymphoma-2 (Bcl-2)-associated X (Bax) [51], Bcl-2-interacting mediator of cell death (Bim) [65], and Bcl-2-homology domain 3 (BH3)-interacting protein (Bid) [64], and inhibits their proapoptotic effects (Fig. 3) [64]. HN is also reported to bind to insulin-like growth factor binding protein 3 (IGFBP-3) and regulates cell survival [65]. Besides the above, HN is shown to bind to actinin 4 [73], a tripartite motif protein TRIM11 [63], and M-phase phosphoprotein 8 (MPP8) [74]. Extracellularly, HN binds to human G protein-coupled formyl peptide receptor-like-1 (FPRL-1) and its murine counterpart FPRL-2 [61]. Of note, FPRL-1 and FPRL-2 are also functional receptors for Amyloid β (Aβ) 42 [61]. HN also binds to a tripartite cytokine-like receptor complex comprising the ciliary neurotrophic factor (CNTF) receptor, the IL-27 receptor WSX1, and glycoprotein (gp) 130 [62]. Activation of these receptors upregulate the Janus Kinase (JAK) 2 and STAT-3 pathways (Fig. 3) [62].

4.1.2. HN mimetics/analogs—The most prominent finding in the structure-function analyses of HN is the substitution of Gly for the 14th Ser residue (HN-$S^{14}G$), which enhances the neuroprotective activity (10 nM for HN-$S^{14}G$ versus 10 μM for HN) by 1000-fold [48]. Comparable neuroprotective efficacy was achieved when L-Ser^{14} was substituted to D-Ser^{14} [75]. The hydrophobic structure of HN made by Leu^{12} to Ile^{16} was reported to be disrupted when Gly or D-Ser was substituted for Ser^{14}, which was implicated for their enhanced potencies [75,76]. HN-$S^{14}G$ has also been reported to be more stable than HN [77]. The third HN mimetic constitute a substitution of Phe in the 6th position with Ala (HN-F^6A), which changes the binding of HN to IGFBP-3 and enhances its main effect on glucose metabolism and insulin sensitivity [67]. The fourth HN mimetic HN-F^6A-$S^{14}G$ (where F^6 was changed to A and S^{14} was changed to G) was found to reduce atherosclerotic plaque size in the proximal aorta of ApoE deficient mice [78].

4.1.3. HN regulation of type 2 diabetes (T2D)—T2D, a heterogenous disease caused by an interaction between genetics (non-modifiable) and environmental (modifiable) factors, increase the risk for insulin resistance, β-cell dysfunction, obesity and ultimately leads to the development of T2D [79-84], which is the most common metabolic disease. T2D is associated with mitochondrial dysfunction (dysregulation of glucose homeostasis and derangement of metabolism) and oxidative stress (caused by hyperglycemia-induced generation of ROS) [85,86]. HN and its mimetic play a significant role in the mitigation of T2D.

4.2. Ex-vivo studies in pancreatic islets

Since impaired glucose-stimulated insulin secretion (GSIS) has been reported in islets obtained from diabetic mice and humans [87,88], the HN mimetic HN-F^6A-$S^{14}G$ was tested for its effect on GSIS in isolated islets from 3-mos-old wild-type (WT) and *db/db* diabetic mice. The exposure of islets to 16 mM glucose plus HN-F^6A-$S^{14}G$ (50, 250, or 500 ng/ml) resulted in augmented insulin secretion (by 3-fold) in islets from WT and 2.5-fold in islets

from diabetic mice [89], indicating that HN mimetic HN-F^{6}A-S^{14}G potentiates GSIS in diabetic mice.

4.3. In vitro studies in mouse βTC3 cells

Like in pancreatic islets, treatment of βTC3 cells (derived from transgenic mice carrying a hybrid insulin promoter-simian virus-40 tumor antigen gene) with 16 mM glucose plus HN-F^{6}A-S^{14}G (50 ng/ml) caused > 2-fold increase in insulin secretion [89]. HN-F^{6}A-S^{14}G-induced insulin secretion was evident after 60 min exposure to 16 mM glucose, which coincided with HN-F^{6}A-S^{14}G-induced ATP production. The authors did not explain why it took 60 min for HN-^{F6A}-S^{14}G to induce secretion of insulin. Mitochondrial membrane potential was not affected by treatment with HN-F^{6}A-S^{14}G [89].

4.4. In vivo studies in rodents

Intracerebroventricular (ICV) administration of HN in 3-mo-old Sprague-Dawley rats under basal insulin levels (~1.41 ng/ml) during pancreatic-euglycemic clamp studies was reported to cause a significant increase in glucose infusion rate (GIR) to maintain euglycemia [90]. The authors believed that increased GIR was due to enhanced hepatic insulin sensitivity owing to decreased HGP.

Under physiologic hyperinsulinemic clamp conditions (insulin levels 3.9 to 4.6 ng/ml), ICV HN into the third ventricle was reported to cause a significant increase in GIR, which was secondary to suppression of HGP (62% in controls vs ~82% in HN-infused group) and associated with enhanced uptake of glucose in skeletal muscle, which the authors implicated an overall improvement in peripheral insulin sensitivity [90]. The authors reported that HN increased phosphorylation of the insulin sensitive AKT (pAKT^{S473}) and Acetyl-CoA Carboxylase (pACC^{Ser79}) in skeletal muscle and phosphorylation of STAT-3 (pSTAT-3 Tyr705) in the hypothalamus, the latter was believed by the authors to be critical for the effects of HN on glucose metabolism [90].

Intravenous administration (at a rate of 0.375 μg/g/hr) of a potent HN mimetic HN-F^{6}A-S^{14}G during a hyperinsulinemic clamp resulted in a significant increase in GIR accompanied with increased glucose uptake in muscle and suppression of HGP. The effects of HN-F^{6}A-S^{14}G on GIR and GSIS were also tested in 3-mo-old male Sprague-Dawley rats in a hyperglycemic clamp study where rats were subjected to 2 h of moderate hyperglycemia (11 mM) followed by glucose infusion for 2 h to maintain the above hyperglycemia. Rats received 20 μg of HN-F^{6}A-S^{14}G as a bolus injection followed by continuous infusion at the rate of 0.07 μg/g/h over 2 h. Under this condition, HN-F^{6}A-S^{14}G caused a moderate increase in GIR (~30%) and a ~2-fold increase in insulin level during the last hour of the clamp [89].

4.5. Studies in humans

Gestational diabetes mellitus (GDM) is defined as hyperglycemia during pregnancy and reflects an early stage of T2D [91,92]. Plasma HN level was reported to be significantly lower in women with GDM, where the HN level was negatively correlated with weight, body-mass index (BMI), and HOMA-IR and might serve as a predictor for the diagnosis of GDM [93]. Like GDM, T2D patients with or without complications also show decreased

levels of plasma HN [94]. In primary human hepatocytes, HN was reported to prevent palmitate-induced hepatic lipid accumulation, and insulin resistance (IR) via AMPK-mediated suppression of the mammalian target of rapamycin (mTOR)/ sterol regulatory element-binding protein (SREBP1) pathway [95].

Polycystic ovary syndrome (PCOS), an endocrine disorder, is characterized by hyperandrogenism and IR [96,97]. HN was reported to be downregulated in the ovaries of PCOS patients with IR as compared to patients without IR [98].

A 12-week resistance training intervention (three times with 60 min/ session/week for 12 weeks) has been reported to cause ~32% increase in HN in skeletal muscle in a male population with impaired glucose regulation [99]. The authors, however, did not find any change in serum HN levels after the above intervention. It has also been reported that patients with impaired fasting glucose had decreased levels of HN protein in plasma compared to a healthy control group [100].

4.5.1. HN regulation of Type 1 diabetes (T1D)—Type I Diabetes (T1D), an autoimmune disorder, is characterized by infiltration of immune cells (T cells and macrophages) which release cytokines like IL-β, IFN-γ, TNF-α during this autoimmune response and are important mediators of destruction of pancreatic β-cells [101,102]. Alterations in mitochondrial electron transport [103], mitochondrial reactive oxygen species [103], mitochondrial nitric oxide [104,105], and mitochondrial hyperpolarization of β-cells [106] are also critical for the destruction of pancreatic β-cells, implicating a link between mitochondria and T1D. It is becoming increasingly evident that apoptosis is the principle cause of β-cell death in the development of T1D [107]. Furthermore, evidences indicate β-cell loss by apoptosis after islet graft [108,109].

It has been reported that HN dose-dependently (1 to 1000 nM) protected (by 50%) NIT-1 insulinoma cells from serum starvation (24 h)-induced apoptosis [110]. The authors have shown abolition of the protective effect of HN after co-treatment with a specific STAT3 inhibitor, which implied STAT3 as a crucial player in this anti-apoptotic effect. HN (1000 nM) was also shown to reduce IFNγ (5 ng/ml) and TNFα (5 ng/ml)-induced apoptosis in NIT-1 cells [110]. In humans, plasma HN levels were reported to be elevated in T1D men compared to T1D women [111].

4.5.2. HN regulation of Alzheimer's disease (AD)—Alzheimer's disease (AD), a leading cause of dementia around the globe [112], is characterized primarily by the extracellular deposition of amyloid β (Aβ) plaques and intracellular neurofibrillary tangles [113-115]. The disease clinically presents with a slow progression of cognitive and behavioral impairment that severely affects day-to-day life [116,117]. Exposure of primary mouse cortical neurons with $A\beta_{1-42}$ resulted in 70–80% death of neurons within 72 h as compared to 20–30% death of neurons in non-treated control cells. Treatment of $A\beta_{1-42}$ exposed primary mouse cortical neurons with HN (10 μm) or its mimetic HN-$S^{14}G$ (10 nM) completely prevented the death of cortical neurons [60]. The authors found that the peptide domain from Pro^3 to Pro^{19} was responsible for neuroprotective action of HN, in which seven residues (Pro^3, Leu^9, Leu^{12}, Thr^{13}, Ser^{14} and Pro^{19}) turned out to be essential [60]. Leu^9

was later identified as the only amino acid residue that is essential in secretion, dimerization, and maintenance of the intact neuroprotection core domain Pro^{3}-Pro^{19} [59]. It is becoming increasingly evident that formyl peptide receptor 2 (FPR2) serves as a receptor mediating the $A\beta_{1–42}$-elicited proinflammatory responses that are implicated in the pathogenic process of AD. FPR2 was identified as a functional receptor of HN and the competitive binding of HN and FPR2 is attributed to the neuroprotective action of HN [118].

It was reported that after 3 and half months of intranasal treatment of 3xTg-AD mice (harboring APP_{swe}, tau^{P301L}, and PS-1^{M146V}) with HN-$S^{14}G$ (10 nmol, 5 days a week), male mice showed significant improvement in spatial learning and memory [119]. In the Morris water maze test, HN-$S^{14}G$-treated mice showed significant difference between platform quadrant and opposite quadrant. The time spent in the area within 60 cm from the platform location was significantly longer for HN-$S^{14}G$-treated male mice than for the vehicle-treated control mice, suggesting better cognitive and memory function HN-$S^{14}G$-treated mice than control [119].

HN-$S^{14}G$ was also reported to ameliorate amnesia caused by muscarinic receptor antagonist [120-122].

4.5.3. HN regulation of healthy aging and lifespan—Aging leads to senescence, or a breakdown of biological processes. In addition, aging exhibits an incapacity to respond to metabolic stress [123] and is deeply associated with the accumulation of mtDNA mutations and the resultant metabolic dysfunction [124,125]. Evidences indicate that serum levels of HN negatively correlates with age and aging-associated diseases like T2D [94,126], AD [127] and cardiovascular diseases [128-130], indicating that supplementation of aging organisms with HN is expected to increase lifespan. Thus, HN-overexpressed transgenic worms experienced a small but significant lifespan expansion [131]. HN promotes healthy aging and increases lifespan by the following mechanisms: (i) by increasing lean body mass and reducing visceral fat [132]; (ii) by promoting the expression of antioxidant defense system proteins by reducing oxidative stress induced by H_2O_2; (iii) by reducing ROS production; (iv) by restoring chaperone-mediated autophagy in cardiomyocytes and cardiac mitochondria [133-135]; (v) by preserving cardiac function after myocardial infarction in an ischemia-reperfusion injury model by reducing cardiomyocyte cell death and myocardial infarct area [51,136, 137]; (vi) by decreasing macrophage infiltration and inflammation, as well as apoptosis, by interacting with the gp130 subunit of the IL-6 receptor, leading to a reduced in vitro production of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α [138-141].

4.6. Mitochondrial ORF within twelve S rRNA c (MOTS-c)

Consistent with the most common secondary structure in naturally-occurring proteins [142-145], MOTS-c displays an α-helical structure [146], which is amenable to protein folding and protein-protein interactions. Besides structural advantage, MOTS-c interacts with and regulates two kinases that regulate metabolism and age-related diseases such as mechanistic target of rapamycin complex I (mTORC1) [146] and AMP-activated protein kinase (AMPK) [12].

4.6.1. MOTS-c regulation of glucose metabolism—Decreased circulating levels of MOTS-c have been reported in humans suffering from obesity [147], IR [148-150], and T2D [151-153], implicating its potential roles in metabolism. MOTS-c improves glucose tolerance in normal chow diet (NCD)-fed mice during glucose tolerance test, while also improving insulin sensitivity by increasing GIR (by 30%) and insulin-stimulated glucose disposal rate (IS-GDR) as assessed by clamp studies [12]. This is achieved by increased expression and translocation of glucose transporter type 4 (GLUT4) to the plasma membrane in muscle cells [12]. MOTS-c also improves insulin sensitivity in insulin-resistant older male mice by enhancing glucose uptake in soleus muscle [12]. It has been shown that MOTS-c promotes glycolysis by stimulating entry of glucose into cells through AMPK pathway [154]. MOTS-c, however, did not inhibit HGP and do not affect the weight of NCD-fed mice [12]. MOTS-c also reduces D-galactose-induced peripheral lipid accumulation [155] and mitochondrial dysfunction [156], which are key players in the pathophysiology of metabolic disease.

In high fat diet (HFD)-induced obese (DIO) and insulin-resistant mice, MOTS-c prevented hyperinsulinemia and obesity by increasing energy expenditure, markedly reduced fat accumulation in liver, promoted activation of AMPK and expression of GLUT4 in the skeletal muscle [12]. These findings indicate that skeletal muscle is a major target organ of MOTS-c. In addition, hepatotoxicity associated with metformin can be avoided with the use of MOTS-c [157].

4.6.2. MOTS-c regulation of fat metabolism—MOTS-c has been reported to increase β-oxidation of fatty acids to prevent fat accumulation in DIO mice and increase insulin sensitivity by reducing sphingolipid metabolism, monoacylglycerol, and dicarboxylate metabolism [39]. Sphingolipid metabolism is associated with obesity and T2D [158,159]. The important metabolite that MOTS-c decreases in DIO mice is sphingosine 1-phosphate (S1P), which inhibits insulin-mediated AKT signaling in the liver and muscle via S1P receptor [160]. However, in muscle, S1P increases expression of interleukin 6 (IL-6), which inhibits insulin-stimulated activation of insulin receptor substrate (IRS) [161]. Monoacylglycerol and dicarboxylate metabolites were significantly decreased in response to MOTS-c. MOTS-c-induced decrease in monoacylglycerol metabolites included 2-Oleoylglycerol, 1-Linoleolylglycerol, 2-Linoleolylglycerol, and 1-Linolenoylglycerol. Likewise, the dicarboxylate metabolism that were reduced by MOTS-c included the following: Suberate, Sebacate, Undecanedioate, and Tetradecanedioate.

4.6.3. MOTS-c modulation of methionine-folate cycle by activation of AMPK —Folate (vitamin B9) and methionine cycles constitute one-carbon metabolism that are linked by methionine synthase, which is a rate-limiting enzyme that converts homocysteine to methionine using 5-methyltetrahydrofolate (5ME-THF) as a methyl donor and B12 as an essential co-factor [162,163]. 5ME-THF, the most abundant form of activated folate, is dramatically decreased in stably overexpressing MOTS-c cells as well as in response to exogenous treatment with synthetic MOTS-c [12]. Decreased 5ME-THF is associated with 20-fold increase in accumulation of AICAR (5-aminoimidazole-4-carboxamide ribonucleotide), which activates AMPK [164] and stimulate fatty acid oxidation via

phosphorylation-induced inactivation of acetyl-CoA carboxylase (ACC) [12]. Inactivation of ACC results in alleviation of allosteric inhibition of carnitine palmitoyltransferase 1 (CPT-1), which is essential for β-oxidation of long chain fatty acids. Activated AMPK increases glucose transporter GLUT4 and the consequent enhancement of glucose uptake in muscle [12,165,166]. Increased phosphorylation of AMPKα (Thr172), ACC (Ser79) and AKT (Ser473), and elevated CPT-1 protein levels by MOTS-c support the above findings.

4.6.4. Nuclear translocation of MOTS-c and regulation of gene expression —MOTS-c rapidly translocates to the nucleus in response to metabolic stress such as, glucose restriction, serum deprivation, and oxidative stress [167]. Nuclear translocation is transient as MOTS-c switches back to being majorly extra-nuclear within 24 hr. Prevention of this nuclear translocation by inhibition of AMPK activity using compound C and siRNA against AMPKα implicates crucial role of AMPK in nuclear translocation of MOTS-c [167]. Furthermore, it has been shown that the hydrophobic domain of MOTS-c (8YIFY11) is required for its nuclear translocation [167]. Upon translocation to the nucleus, MOTS-c directly binds to the DNA sequences of antioxidant response element (ARE) containing promoter regions (5'-TGACNNNGC-3') of nuclear factor erythroid 2-related factor 2 (*NRF2*) target genes, including Heme Oxygenase 1 (*HMOX1*), NAD(P)H Dehrdrogenase Quinone 1 (*NQO1*), UDP-Glycosyltransferase 1 Family Polypeptide A1 (*UGT1A1*), UDP-Glycosyltransferase 1 Family Polypeptide A6 (*UGT1A6*), Thioredoxin (TXN), Ferritin Light Chain (*FTL*), and Glutathione Peroxidase 2 (*GPX2*). Of note, NRF2 intersects with AMPK [168] and regulate MOTS-c-related metabolic pathways [169].

4.6.5. MOTS-c regulation of aging and exercise—Aging leads to senescence, or a breakdown of biological processes. In addition, aging exhibits an incapacity to respond to metabolic stress [123] and is deeply associated with the accumulation of mtDNA mutations and the subsequent metabolic dysfunction [124,125]. Like obesity [147], IR [148-150], and T2D [151-153], MOTS-c levels also decrease with age in humans. Middle-aged (45–55 years) and old-aged (70–81 years) individuals display 11% and 21% lower circulating MOTS-c levels compared to younger individuals (18–30 years), respectively [170]. Like humans, aged mice (4 mo vs. 32 mo) also show decreased plasma MOTS-c [12]. Unlike rodents, the levels of MOTS-c in skeletal muscle of the elderly humans were the highest, indicating the levels of MOTS-c in plasma and muscle decrease gradually with age [170]. These findings implicate higher levels of MOTS-c is beneficial to delaying aging.

Oxidized nicotinamide adenine dinucleotide (NAD^+), and its reduced form, reduced nicotinamide adenine dinucleotide (NADH), are critical molecules as they support various metabolic functions [171-174]. As a co-enzyme, NAD^+ catalyzes cellular redox reactions, and gets reduced to NADH, in many metabolic processes, such as glycolysis, fatty acid beta oxidation, or the tricarboxylic acid cycle [175-177]. NAD^+ also acts as a co-substrate for three classes of enzymes: (i) the sirtuins (SIRTs), (ii) the adenosine diphosphate (ADP)-ribose transferases (ARTs) and poly(ADP-ribose) polymerase (PARPs), and (iii) the cyclic ADP-ribose (cADPR) synthases (CD38 and CD157) [178,179]. Since the NAD^+/NADH ratio declines with aging in worms, mice, and humans [180,181], restoring NAD^+ levels can improve age-related disease conditions [174,179]. MOTS-c extends mouse lifespan by

increasing NAD^+ levels and activating glycolytic effects via sirtuin 1 (SIRT1) [182]. In addition, MOTS-c restricts the folate/methionine cycle, causing a reduction in methionine metabolism. In rodents, methionine shortage can extend lifespan by 45%, lowers visceral fat and age-related diseases (e.g., cancer), and increases the major antioxidant glutathione (GSH) [183-186].

Exercise is one of the interventions that prevents age-related adverse effects in mice and humans [187-193]. Intraperitoneal administration of MOTS-c has been reported to improve the physical performance of mice of different ages (2, 12, 22 and 23.5 months) and slowed the emergence of age-related deficits over a two-week period [194,195]. Of note, MOTS-c treatment upregulated glycolytic and protein metabolism markers following exercise and led to an enrichment of genes associated with protein regulation/metabolism, cellular metabolism, and oxidative stress response [195].

4.6.6. Association of single nucleotide polymorphism in MOTS-c with T2D

—It has been shown that 5–10% of people with East Asian descent have a non-synonymous mitochondrial DNA polymorphism in the MOTS-c coding region, m.A1382C (rs111033358), that causes an amino acid replacement from Lys (K) to Gln (Q) at the 14th amino-acid residue [123]. Meta-analysis with three cohorts (n = 27,527) including Japan multi-institutional collaborative cohort (J-MICC, Saga City), multiethnic cohort (Hawai and California), and Tohoku medical megabank project (Pacific coast of the Tohoku region in Japan) showed that men with C allele of m.A1382C exhibit a significantly higher risk of T2D [152]. Of particular interest, in J-MICC, in sedentary males with C allele of m. A1382C showed higher prevalence of T2D, demonstrating a kinesio-genomic interaction [152]. Treating HFD-fed mice with $K^{14}Q$ MOTS-c has been reported not to confer the metabolic benefits associated with native MOTS-c administration [152]. In vitro and in vivo studies confirm that MOTS-c $K^{14}Q$ is a partially bioinactive form of MOTS-c peptide [152]. Other mtDNA polymorphisms that contribute to T2D risk include *MTND1* T4216C and *MTND2* $A^{4917}G$ in European population [196], and N9a haplogroup in Asian (Japanese and Korean) population [197].

4.7. SHLP1

Immunoblotting revealed high expression of SHLP1 in mouse heart, kidney, and spleen [11]. Liver, brain, prostate, testis, and muscle also express significant amounts of SHLP1. Preliminary studies on murine derived NIT-1 murine β-cells and human prostate cancer cells 22Rv1 revealed that SHLP1 had no effect on cellular viability [11]. Its specific roles remain to be deciphered and a scope of further studies remain in delineating its exact physiological role.

4.8. SHLP2

Immunoblotting showed highest expression of SHLP2 in mouse liver, kidney, and muscle [11]. Plasma SHLP2 levels were higher in males than in females.

4.8.1. SHLP2 regulation of obesity and insulin sensitization

4.8.1.1. Peripheral actions on obesity, thermogenesis, and insulin sensitivity.: Obese and diabetic patients as well as murine models of obesity and diabetes such as *ob/ob* and *db/db* mice show decreased serum levels of SHLP2 [198], implicating that supplementation of obese mice with SHLP2 would improve insulin sensitivity. Thus, three weeks of treatment of DIO mice with intraperitoneal SHLP2 (2 μg/g body weight, once daily) caused the following phenotypes: (i) protection of mice against diet-induced increase in body weight, (ii) decrease in total body fat mass and circulating leptin levels, (iii) reduction in the size of inguinal and epididymal white adipose tissues, and (iv) profound decrease in HFD-induced hepatic steatosis [198]. Furthermore, SHLP2 treatment resulted in a significant lowering of blood glucose levels and improved glucose tolerance and sensitivity [198]. SHLP2 in presence of insulin promoted differentiation of 3T3-L1 murine pre-adipocytes, indicating its insulin-sensitizing effect in adipose tissue [11]. SHLP2 not only enhanced cell viability and decreased apoptosis in both NIT-1β and 22Rv1 cells but promoted cell proliferation in NIT-1β cells. Furthermore, SHLP2 treatment of C57BL/6 mice (2 μg/g body weight, BID, IP) for 5 days though had no significant effects on plasma insulin, IL-6, or monocyte chemoattractant protein-1 (MCP-1), but increased plasma leptin level without affecting body weight and food intake [11].

In addition, systemic SHLP2 administration also caused robust increase in O_2 consumption (VO_2), CO_2 production (VCO_2) as well as heat generation by increasing expression of the genes involved in inguinal brown adipose tissue (iBAT) thermogenesis including peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (*Pgc1α)*, iodothyronine deiodinase 2 (*Dio2)*, PR domain-containing protein 16 (*Prdm16*), and nuclear respiratory factor 1 (*Nrf1)*. SHLP2 also showed a significant reduction in daily food intake [198], which was attributed to a reduction in the expression of orexigenic neuropeptides in the hypothalamus such as agouti-related peptide (*Agrp*) and neuropeptide Y (*Npy*). Of note, SHLP2 was shown to cross the blood-brain barrier.

4.8.1.2. Peripheral effects on sphingolipid metabolism.: Metabolomic studies in DIO mice after three days of treatment with SHLP2 (2.5 μg/g body weight; twice daily) revealed significant alterations in the concentrations of lipid metabolites in plasma [199]. Since feeding HFD increases sphingomyelin levels in liver, adipose tissue and plasma, SHLP2's effects were tested in DIO mice, which showed significant decrease in plasma levels of sphingolipids such as sphinganine, sphingomyelin, sphinganine-1-phosphate, glucosyl N-stearoyl sphingosine, and glycosyl N-palmitoyl sphingosine [199].

4.8.1.3. Peripheral effects on mitochondrial metabolism.: In human prostate cancer cells (22Rv1), SHLP2 caused significant increase in mitochondrial oxygen consumption rate (OCR) and cellular ATP, indicating enhanced mitochondrial metabolism by SHLP2. Pre-incubation of murine β-cells (NIT-1) and 22Rv1 cells with SHLP2 overnight resulted in significant suppression of serum-starvation-dependent formation of ROS, suggesting a cytoprotective role of SHLP2. In addition, SHLP2 fully blocked staurosporine-induced apoptosis in NIT-1β cells.

4.8.2. Central actions of SHLP2 on obesity, thermogenesis, and insulin sensitivity—

Like systemic administration, ICV administration of SHLP2 (3 μg) into third ventricle also protected the male mice from HFD-induced obesity, increased expression of thermogenic genes and uncoupling protein 1 (UCP1) in inguinal brown adipose tissue (iBAT), and improved glucose tolerance [39]. Therefore, it was thought that the thermogenic and anorexigenic effects of SHLP2 might be mediated through the central nervous system (CNS) [39].

Continuous ICV infusion of SHLP2 (at a rate of 0.16 ng/g/min) into conscious Sprague Dawley rats significantly improved insulin sensitivity by increasing GIR, suppressing HGP, and increasing peripheral glucose uptake in hyperinsulinemic-euglycemic clamp studies [11].

4.8.3. SHLP2 regulation of macular degeneration—

Expression of MDP-coding *MT-RNR2* gene along with all five oxidative phosphorylation (OXPHOS) complex I-V protein subunits have been reported to be downregulated in human transmitochondrial age-related macular degeneration (AMD) ARPE-19 cell model. However, treatment of AMD cells with SHLP2 resulted in the following changes: (i) restoration of the normal levels of OXPHOS complex protein subunits, (ii) prevention of loss of viable cells and mitochondria, (iii) induction of anti-apoptotic effects, and (iv) attenuation of amyloid-β-induced cellular and mitochondrial toxicity [200].

4.8.4. SHLP2 regulation of Parkinson's disease (PD)—

The substantia nigra of PD patients show deficiencies of mitochondrial respiratory chain complex I activity [201]. Attenuated risk of PD has been reported in patients carrying mtDNA SNP (m .2158 T > C where lysine 4 is changed to arginine). Further studies revealed that K4R SHLP2 is not only more stable than WT SHLP2, but also more potently inhibited PD toxin (MPP+)-induced apoptosis in neuronal cells [202].

4.8.5. SHLP2 regulation of AD—

Increased $A\beta_{1-42}$ is the hallmark of AD [203]. Exposure of primary cortical neurons with 0.1 or 10 μM SHLP2 prevented $A\beta_{1-42}$-induced neuronal cell death, implicating association of SHLP2 with AD [11].

4.8.6. SHLP2 regulation of prostate cancer—

Since SHLP2 levels were found to be nearly halved in prostate cancer patients, SHLP2 levels are used as a biomarker for cancer patients [204].

4.9. SHLP3

Immunoblotting showed highest expression of SHLP3 in mouse brain, and spleen [11]. Kidney, prostate, and testis also express significant amounts of SHLP3. An immunoassay to measure SHLP3 is yet to be developed.

Like SHLP2, SHLP3 increases cell viability, decreases cellular apoptosis in both NIT-1β and 22Rv1 cells, increases mitochondrial functions by increasing mitochondrial OCR, cellular ATP, and decreasing the ability to produce ROS, implicating its cytoprotective nature [11]. Unlike SHLP2, SHLP3 did not exert insulin-sensitizing effects in vivo. Like SHLP2, SHLP3

increases plasma leptin levels without altering food intake and body weight. As a sharp contrast to SHLP2, SHLP3 increases proinflammatory cytokines IL-6 and MCP-1, which possibly explains the lack of an in vivo insulin-sensitizing effect of SHLP3 [11].

4.10. SHLP4

Immunoblotting showed highest expression of SHLP4 in mouse liver, spleen, and prostate [11]. In addition, brain, kidney, and testis also show detectable amounts of SHLP4. Like SHLP2, SHLP4 also increase cellular proliferation in murine NIT-1 cells.

4.11. SHLP5

SHLP5 is the least studied SHLP till date. Apart from its peptide sequence not much has been elucidated in any scientific study hitherto.

4.12. SHLP6

Immunoblotting showed significant expression of SHLP6 in mouse heart, liver, and kidney [11]. A sharp contrast to SHLP2 and SHLP3, SHLP6 increased apoptosis in both NIT1 and 22Rv1 cells [11,38]. Plasma levels of SHLP6 increased significantly after acute exercise in young human males, which returned to baseline during recovery [205]. Like humanin, short-term high intensity interval training (HIIT) lead to an overall lower plasma concentration of SHLP6 but did not change the response to exercise [205].

5. Newly discovered mitochondrial microproteins

Two mitochondrial microproteins have recently been identified: SHMOOSE (Small Human Mitochondrial ORF Over SErine tRNA; 58 amino acids) [206] and MTALTND4 (mitochondrial alternative ND4 protein; 99 amino acids) [207]. SHMOOSE binds to intermembrane space protein Mic60 (mitofilin) and modifies mitochondrial biology including increase in neural cell metabolic activity (by 10–20%) and increase in basal oxygen consumption rate by ~20%. Furthermore, SHMOOSE expression was found to be high (~15% greater compared control) in AD patient brains, linking its association AD pathophysiology [206]. In addition, treatment of neuronal cells (stressed with oligomerized amyloid beta) with SHMOOSE has been reported to protect those cells from death [206]. Like SHMOOSE, MTALTND4 also modulates mitochondrial function [207].

6. Conclusion and future perspectives

Mitochondria are the “powerhouse of the cell” as they are the main sites of ATP (energy currency of the cell) production [208]. Besides energy production, this important sub-cellular organelle through its MDPs (e.g., MOTS-c) have developed extensive retrograde signaling networks to communicate with the nuclear genome, other intracellular organelles, and potentially neighboring cells or organs [209]. Therefore, mitochondrial dysfunction is believed to be a key player in the pathophysiology of metabolic diseases, including obesity, IR and T2D [156]. Consistent with this hypothesis, it has been reported that T2D patients show reduced $NADH_2$-O_2 oxidoreductase activity (attributed to complex I) and structural mitochondrial aberrations coupled with decreased subsarcolemmal mitochondrial function

[210,211]. Other studies using muscle biopsy samples from patients with T2D and from individuals with a positive family history of T2D revealed downregulation of genes that encode proteins involved in oxidative metabolism [212,213].

Circulating levels of MDPs (HN and MOTS-c) show negative correlation in humans suffering from obesity [147], IR [148-150], T2D [94,100,151-153], and GDM [93], implicating their crucial roles in metabolism. In addition, in humans, plasma levels of MDPs (HN, MOTS-c and SHLP2) show negative correlation with aging and age-related diseases like AD [214,215], indicating their critical roles in promoting lifespan and health. Based on the multifaceted actions, MDPs pave a new conceptual way for the treatment of metabolic (e.g., obesity, steatosis, and T2D) and age-related diseases like AD. Clinical trials to test the therapeutical potential of an MDP, MOTS-c is ongoing but currently limited, including a clinical trial using a MOTS-c analog for fatty liver and obesity (clinical trial #NCT03998514). Further studies are required to better understand the basic molecular mechanisms of MDPs, their stabilities in biological systems, oral bioavailability, and relevance to a broad range of diseases and conditions.

Based on a recent synonymous codon bias study in vertebrates [57], it was revealed that HN and SHLP6 exhibited strong synonymous codon bias and sequence conservation. As a sharp contrast, SHLP1, SHLP2, SHLP3, and SHLP5 showed no significant synonymous codon bias, and the sequences are poorly conserved. Although MOTS-c and SHLP4 lack significant synonymous codon bias, they contain highly conserved N-terminal regions. Sequence homology analyses of MOTS-c in 14 mammalian species revealed the following conservation: M^1, M^6, G^7, and Y^8 (100%); I^9 and F^{10} (~94%); R^2 (~44%); W^3 (~82%); Q^4 (~37%), E^5 (~94%), and Y^{11} (~87%) [12]. In this context, it should be pointed out that like MDPs, sequence alignment of a nuclear-encoded Chromogranin A (CgA) derived peptide Catestatin (CST: human $CgA_{352–372}$) in 53 mammalian species belonging to 8 orders revealed > 80% homology in 52 species, except in Platypus (lowest in the mammalian phylogenetic tree) where the homology with the primates (highest in the mammalian phylogenetic tree was >58%), indicating that CST is also highly conserved in mammals [216-218]. Besides sequence conservation, MDPs and CST exhibit several comparable features: length (MDP: 16–38 amino acids versus CST: 21 amino acids); isoelectric point (MDPs: 8.73 - 12.31 (except SHLP1 and SHLP6) versus CST: 12.03); charge at pH 7.0: (MDPs: 1.22 - 2.83 versus CST: 3.83). In addition to the evolutionary conservation and comparable physicochemical properties, MDPs and CST exhibit comparable anti-diabetic [12,19,151,157,219], anti-oxidative [133,220-222], anti-inflammatory [19,223,224], anti-apoptotic [225,226], cardioprotective [224,227-231], and neuroprotective effects [59,62,232]. Therefore, it is reasonable to assume that retrograde signaling of MDPs can regulate nuclear encoded genes such as CgA to maintain health and diseases.

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Data availability

No data was used for the research described in the article.

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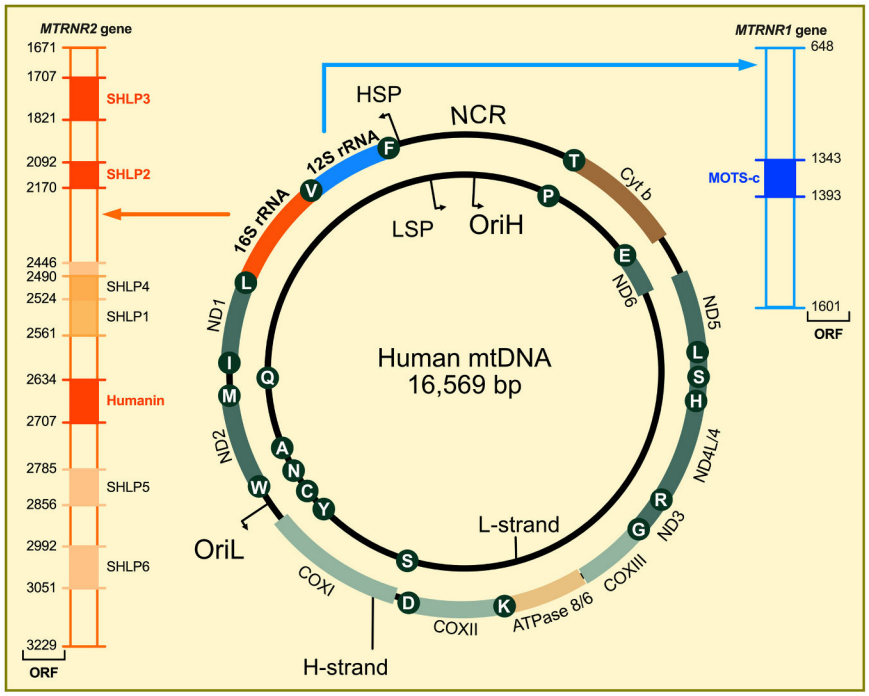

Fig. 1. Map of the human mitochondrial genome.

NCR (non-coding region), and OriL (origin of replication for the light strand) represent the major non-coding regions. The outer and inner circles represent the heavy (H) and light (L) strands, respectively. The G-rich H-strand encode 12 subunits of the OXPHOS system (ND1–3, ND5, Cytb and COI-III, ND4/ND4L and ATP6/ATP8), 2 mtRNAs (12 S and 16 S), and 14 mt-tRNAs (F, V, L, I, M, W, D, K, G, R, H, S, L, T). The L-strand encode only 1 mitochondrial messenger RNA (mtRNA) that encodes subunit 6 of NADH dehydrogenase (ND6), and 8 mt-tRNAs (Q, A, N, C, Y, S, E, and P). *MTRNR2* gene and the encoded peptides including humanin and small humanin-like peptides (SHLPs) such as SHLP1, SHLP2, SHLP3, SHLP4, SHLP5, and SHLP6 are shown on the left. *MTRNR1* gene and the encoded peptide MOTS-c are shown on the right.

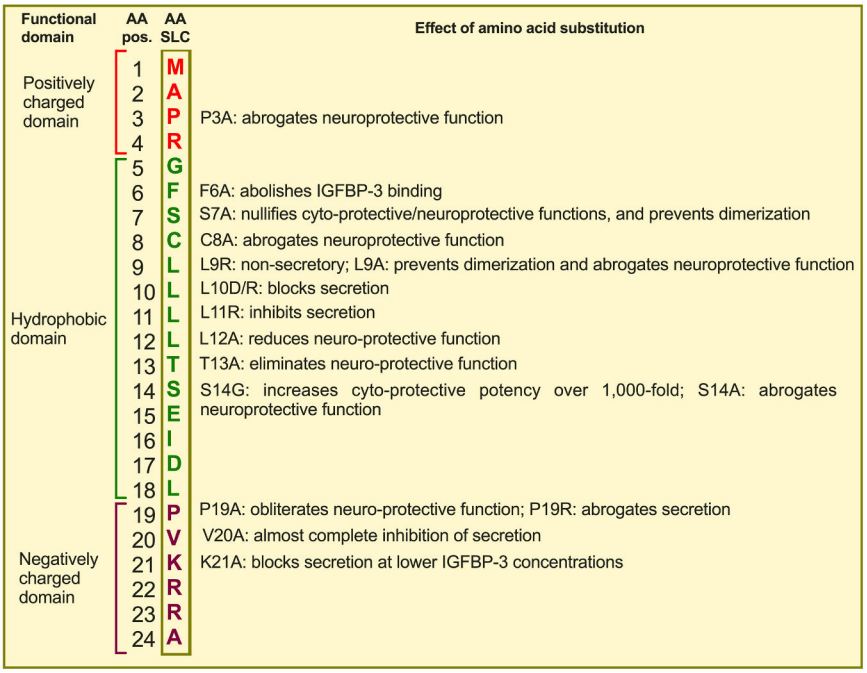

Fig. 2.

Functional domains in the primary structure of Humanin and the effects of amino acid substitution on the function of Humanin. AA pos: amino acid position; AA SLC: amino acid single letter code.

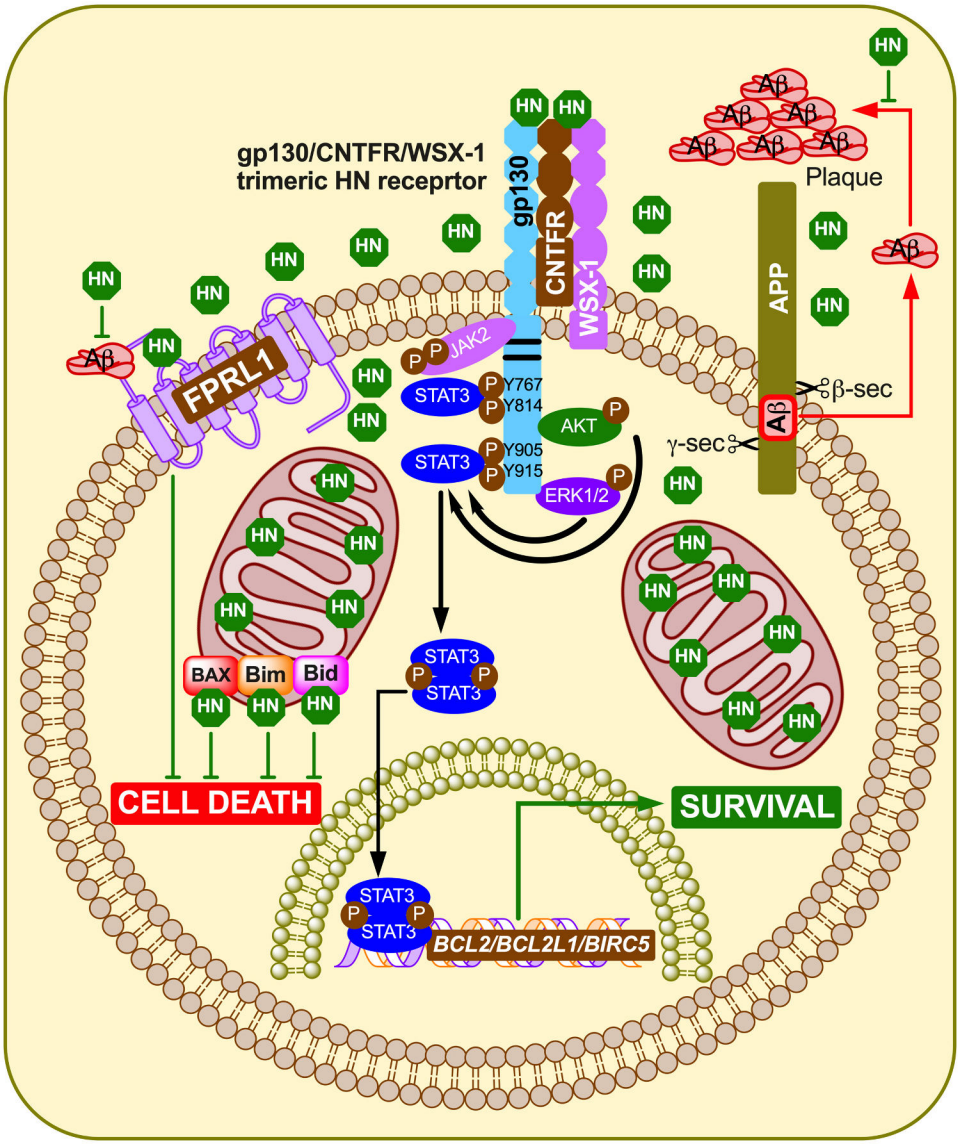

Fig. 3. Binding partners, putative receptors, and intracellular signaling for HN.

Intracellularly, HN binds with Bax, Bim, and Bid, and inhibits their proapoptotic effects. Extracellularly, HN binds to two cell surface receptors: formylpeptide receptor like-1 (FPRL-1) and heterotrimeric HN receptor (htHNR) comprising of WSX-1, a subunit for cytokine IL-27, ciliary neurotrophic receptor a subunit (CNTFR), and gp130. FPRL-1 serves as a receptor for both HN and Ab. HN inhibits Ab-induced cell death by competitively inhibiting the binding of Ab to FPRL-1. HN activates JAK2/STAT3-mediated pro-survival signaling by binding to htHNR.

Table 1

Physicochemical properties of MDP and Catestatin (CST).

Gene	Peptide	Length (amino acids)	Molecular weight	Extinction co-efficient	Absorbance (280 nm, 0.1%)	Isoelectric point (pI)	Charge at pH 7.0
MTRNR2	Humanin	24 aa	2687.27 Da	0 M^{-1} cm^{-1}	0.00	9.12	1.22
MTRNR1	MOTS-c	16 aa	2174.62 Da	8480 M^{-1} cm^{-1}	3.9	10.63	2,83
MTRNR2	SHLP1	24 aa	2393.61 Da	5500 M^{-1} cm^{-1}	2.3	6.64	−0.49
MTRNR2	SHLP2	26 aa	3017.55 Da	5500 M^{-1} cm^{-1}	1.82	12.31	2.83
MTRNR2	SHLP3	38 aa	4380.16 Da	15,470 M^{-1} cm^{-1}	3.53	8.73	1.22
MTRNR2	SHLP4	26 aa	3131.87 Da	0 M^{-1} cm^{-1}	0.00	11.13	2.22
MTRNR2	SHLP5	24 aa	2565.97 Da	1490 M^{-1} cm^{-1}	0.58	3.42	−4.39
MTRNR2	SHLP6	20 aa	2385.88 Da	0 M^{-1} cm^{-1}	0.00	3.93	−1.17
CHGA	CST	21 aa	2326.71 Da	1490 M^{-1} cm^{-1}	0.64	12.03	3.83

Table 2

Synonymous codon bias in humanin.

Amino acid number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
Human sequence	**M**	**A**	**P**	**R**	**G**	**F**	**S**	**C**	
Codon bias in Primates (n = 252)		**0.99**	0	0	0.33	**0.54**	0	*	
Codon bias in Mammals (n = 148)		**0.98**	0	0	0.45	**0.63**	0	*	
Codon bias in Vertebrates (n = 359)		**0.91**	0	0	**0.63**	0.11	0	*	
Amino acid number	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	
Human sequence	**L**	**L**	**L**	**L**	**T**	**S**	**E**	**I**	
Codon bias in Primates (n = 252)	**1.0**	**0.72**	0.40	0.09	0	0	*	0	
Codon bias in Mammals (n = 148)	**1.0**	**0.57**	0.32	0.05	0	0	0	0	
Codon bias in Vertebrates (n = 359)	**1.0**	**0.70**	0.09	0.09	0.01	0.01	0	0	
Amino acid number	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**Ter**
Human sequence	**D**	**L**	**P**	**V**	**K**	**R**	**R**	**A**	**Ter**
Codon Primates	0.20	**0.93**	0.02	0	0.44	**1.0**	**0.91**	0	0.24
Mammals	0.20	**0.86**	0.22	0	0.08	**0.86**	0.35	0	0.19
Vertebrates	**0.86**	**0.85**	0.43	**0.85**	0.33	0.06	0.23	0	0.17

Table 3

Synonymous codon bias in MOTS-c.

Amino acid number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Human sequence	**M**	**R**	**W**	**Q**	**E**	**M**	**G**	**Y**	**I**
Codon bias in Primates (n = 252)		0.26		0	0.09	*	*	*	*
Codon bias in Mammals (n = 148)		0.21		0	0.29	*	**0.73**	0	*
Codon bias in Vertebrates (n = 359)		0.14		0	0.30		0.14	0	0
Amino acid number	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**Ter**	
Human sequence	**F**	**Y**	**P**	**R**	**K**	**L**	**R**	**Ter**	
Codon bias in Primates (n = 252)	0.13	0.32	0.02	0.01	0	0.09	0	0.17	
Codon bias in Mammals (n = 148)	0	0.24	0.10	0.05	0	0.09	0.12	0.02	
Codon bias in Vertebrates (n = 359)	0.07	0.17	0.08	0.17	0.07	0.13	0.08	0.03	

Table 4

Synonymous codon bias in SHLP2.

Amino acid number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Human sequence	**M**	**G**	**V**	**K**	**F**	**F**	**T**	**L**	**S**
Codon bias in Primates (n = 219)		0.31	0.14	0.08	0.18	0	0	0.41	0.43
Codon bias in Mammals (n = 174)		0.38	0.14	0.06	0.09	0.05	0.01	0.11	0.08
Codon bias in Vertebrates (n = 369)		0.19	0.11	0.06	0.17	0.02	0.01	0.11	0.07
Amino acid number	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Human sequence	**T**	**R**	**L**	**F**	**P**	**S**	**V**	**Q**	**R**
Codon bias in Primates (n = 219)	0.13	0.05	0	0.07	0.04	0.01	0.06	0.05	0.05
Codon bias in Mammals (n = 174)	0.05	0.03	0.09	0.11	0.13	0.06	0.06	0.03	0.17
Codon bias in Vertebrates (n = 369)	0.14	0.04	0.09	0.09	0.06	0.09	0.12	0.02	0.12
Amino acid number	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**Ter**
Human sequence	**A**	**V**	**P**	**L**	**W**	**T**	**N**	**S**	**Ter**
Codon bias in Primates (n = 219)	0	0.20	0.26	**0.56**	0.06	0	0	0	0.17
Codon bias in Mammals (n = 174)	0	0.29	0.21	0.26	0.06	0.13	0.01	0	0.02
Codon bias in Vertebrates (n = 369)	0.13	0.16	0.08	0.15	0.04	0.16	0.06	0.13	0.20

Table 5

Synonymous codon bias in SHLP4.

Amino acid number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Human sequence	**M**	**L**	**E**	**V**	**M**	**F**	**L**	**V**	**N**
Codon bias in Primates (n = 215)		**1.0**	0	*	*	*	0	0	0
Codon bias in Mammals (n = 144)		**0.91**	0	**0.50**	*	*	*	*	*
Codon bias in Vertebrates (n = 339)		0.39	0.03	0.29		*	0	0	0
Amino acid number	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Human sequence	**R**	**R**	**G**	**K**	**I**	**C**	**R**	**V**	**P**
Codon bias in Primates (n = 215)	0	**0.50**	**0.96**	0.27	0.01	0.01	0.31	0	0
Codon bias in Mammals (n = 144)	*	**0.89**	0.42	0.22	0.04	0	0.06	0	0.03
Codon bias in Vertebrates (n = 339)	0.01	**0.88**	**0.53**	0.27	0.01	0.02	0.22	0	0.24
Amino acid number	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**Ter**
Human sequence	**F**	**T**	**F**	**F**	**N**	**L**	**S**	**L**	**Ter**
Codon bias in Primates (n = 215)	0	0.01	0.19	0.10	0.07	0	0.07	**0.78**	0.07
Codon bias in Mammals (n = 144)	0.36	0.29	**0.52**	0.06	0.09	0	0.13	0.42	0.08
Codon bias in Vertebrates (n = 339)	**0.52**	0.09	0.25	0.16	0.26	0.07	0.18	0.22	0.07

Table 6

Synonymous codon bias in SHLP6.

Amino acid number	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Human sequence	**M**	**L**	**D**	**Q**	**D**	**I**	**P**
Codon bias in Primates (n = 242)	*	*	*	**0.99**	**1.0**	0	0.10
Codon bias in Mammals (n = 147)	*	*	*	**0.88**	**1.0**	0	0.22
Codon bias in Vertebrates(n = 348	*	*	**1.0**	**0.62**	0.43	0	0.19
Amino acid number	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Human sequence	**M**	**V**	**Q**	**P**	**L**	**L**	**K**
Codon bias in Primates(n = 242)	0	*	0.41	0	**1.0**	0.20	0
Codon bias in Mammals(n = 147)		*	**0.59**	0.01	*	0.07	0
Codon bias in Vertebrates(n = 348)		*	**0.55**	0.02	**0.73**	0.35	0
Amino acid number	**15**	**16**	**17**	**18**	**19**	**20**	**Ter**
Human sequence	**V**	**R**	**L**	**F**	**N**	**D**	**Ter**
Codon bias in Primates(n = 242)	**0.91**	0	*	*	0	0	*
Codon bias in Mammals(n = 147)	0.20	0	0	0	0	0	0
Codon bias in Vertebrates(n = 348)	0.09	0	0	0	0	0	0