

Humanin: a harbinger of mitochondrial-derived peptides?

Changhan Lee, Kelvin Yen, and Pinchas Cohen

University of Southern California Davis School of Gerontology, Ethel Percy Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089-0191, USA

Mitochondria have been largely considered as ‘end-function’ organelles, servicing the cell by producing energy and regulating cell death in response to complex signals. Being cellular entities with vital roles, mitochondria communicate back to the cell and actively engage in determining major cellular policies. These signals, collectively referred to as retrograde signals, are encoded in the nuclear genome or are secondary products of mitochondrial metabolism. Here, we discuss humanin, the first small peptide of a putative set of mitochondrial-derived peptides (MDPs), which exhibits strong cytoprotective actions against various stress and disease models. The study of humanin and other mitochondrial-derived retrograde signal peptides will aid in the identification of genes and peptides with therapeutic and diagnostic potential in treating human diseases.

Mitochondrial biology and function

It is widely accepted that the endosymbiotic origin of mitochondria was from α -proteobacteria that have been engulfed by and integrated into eukaryotic host cells. The incorporation of the mitochondrion (proto-mitochondrion) has changed the course of eukaryotic evolution through a monumental metabolic upgrade by employing oxygen to mass-produce energy and biosynthetic precursors [1]. Owing to their prokaryotic origin, mitochondria are also unique among intracellular organelles in that they contain their own genome. This small genome is thought to be the result of gradual loss or transfer of the great majority of the original bacterial genes to the nucleus, leaving only 13 full-size protein-coding genes that are essential for oxidative phosphorylation. This component of the human genome is beginning to be explored for its potential role in conditions such as aging, cancer, diabetes, deafness, and neurodegeneration [2].

The mitochondrion is responsible for several crucial cellular activities including energy production, regulation of programmed cell death (apoptosis), biosynthetic precursor production, heme synthesis, Fe–S cluster production, ion homeostasis, and reactive oxygen species (ROS) production [3]. Bestowed with such critical responsibilities, it has been known that mitochondria actively participate in determining cellular processes; however, little is understood about how mitochondria transmit information to the host cell. In this article we will discuss a newly emerging

concept regarding a novel class of communication conveyed by the mitochondrion to regulate cellular processes and cell fate (Figure 1).

The concept of retrograde signaling from the mitochondrion to the nucleus and beyond

Mitochondria have traditionally been perceived as ‘end-function’ organelles that receive cellular signals, and in response regulate energy production and apoptosis. However, a coordinated regulation of mitochondrial and nuclear gene expression is critical for cellular homeostasis, which requires constant and active exchange of information [4]. Mitochondrion-initiated communication events that signal and regulate various cellular aspects under normal, stress, and pathological states are referred to *en masse* as retrograde signaling [4], a biological process well conserved from the simple yeast to humans (Figure 1).

To date, a relatively limited number of retrograde signaling molecules and signaling cascades have been explored. Some of the molecules described in this regard are cytochrome *c*, ROS, Ca^{2+} , Fe^{2+} , nitric oxide (NO), and carbon monoxide (CO) [5–7] (Figure 2). Notably, an interesting study in *C. elegans* described non-canonical signals exported from the mitochondria in response to proteotoxic insults [8]. However, these were not peptides encoded within the mitochondria but instead damaged

Glossary

Membrane permeability transition (MPT): an increase in mitochondrial permeability via the formation of a nonspecific pore across the inner mitochondrial membrane, permitting the free distribution of ions and low molecular weight molecules (<1500 Da) across the membrane that can lead to mitochondrial swelling and cell death.

Mitochondrial-derived peptides (MDPs): small peptides that are encoded within the mitochondrial genome in addition to the canonical 13 protein-coding mitochondrial genes.

Nuclear mitochondrial DNA transfer (NUMT): a copy of mitochondrial DNA (mtDNA) that is integrated into the nuclear genome; these are generally considered to be pseudogenes. Also known as nuclear DNA sequences of mitochondrial origin.

Parallel analysis of RNA ends (PARE): a technique that combines 5′-rapid amplification of cDNA ends (RACE) with high-throughput deep sequencing (SBS) and bioinformatics tools that can reveal RNA cleavage sites.

Retrograde signaling: a pathway of communication from the mitochondria to the cell that influences cellular and organismal activities.

Retrograde signaling molecules: these are ions, proteins, and molecules that act as messengers of retrograde signaling (e.g., Ca^{2+} , reactive oxygen species, cytochrome *c*, etc.).

tRNA punctuation model: a model describing mitochondrial RNA processing, whereby individual genes are released from a long polycistronic transcript by excising the 22 interspersed tRNAs that are strategically encoded in the mtDNA.

Corresponding author: Cohen, P. (hassy@usc.edu).

Keywords: humanin; mitochondria; retrograde signaling; protection; stress-resistance; mitochondrial-derived peptide.

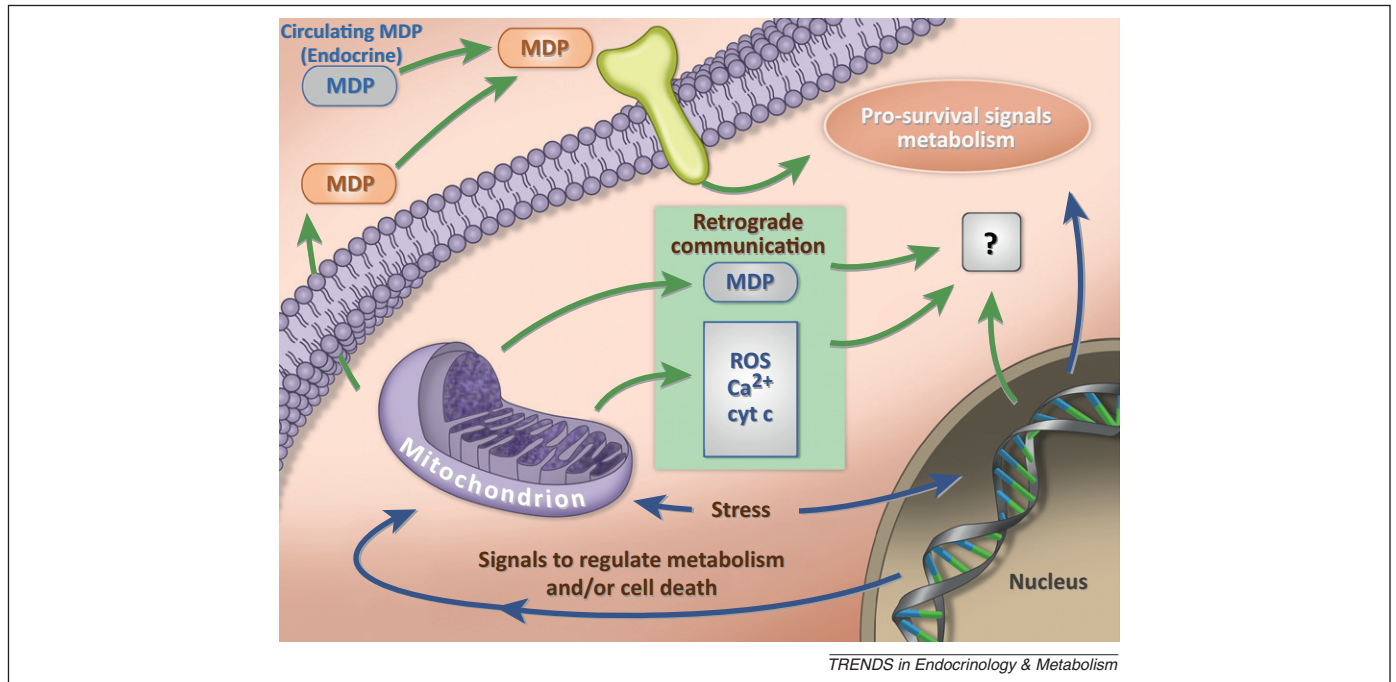


Figure 1. Mitochondrial-derived peptides (MDPs) are retrograde signaling molecules. Mitochondria communicate with the cell through a process largely known as retrograde signaling. Traditionally described means of communication from the mitochondria include Ca^{2+} , cytochrome c (cyt c), and reactive oxygen species (ROS). MDPs are recently identified retrograde signals, which are unique in that they are encoded within mitochondrial genome sequences. MDPs such as humanin are thought to act as endocrine as well as intracellular factors with several biological roles in the regulation of cell survival and metabolism.

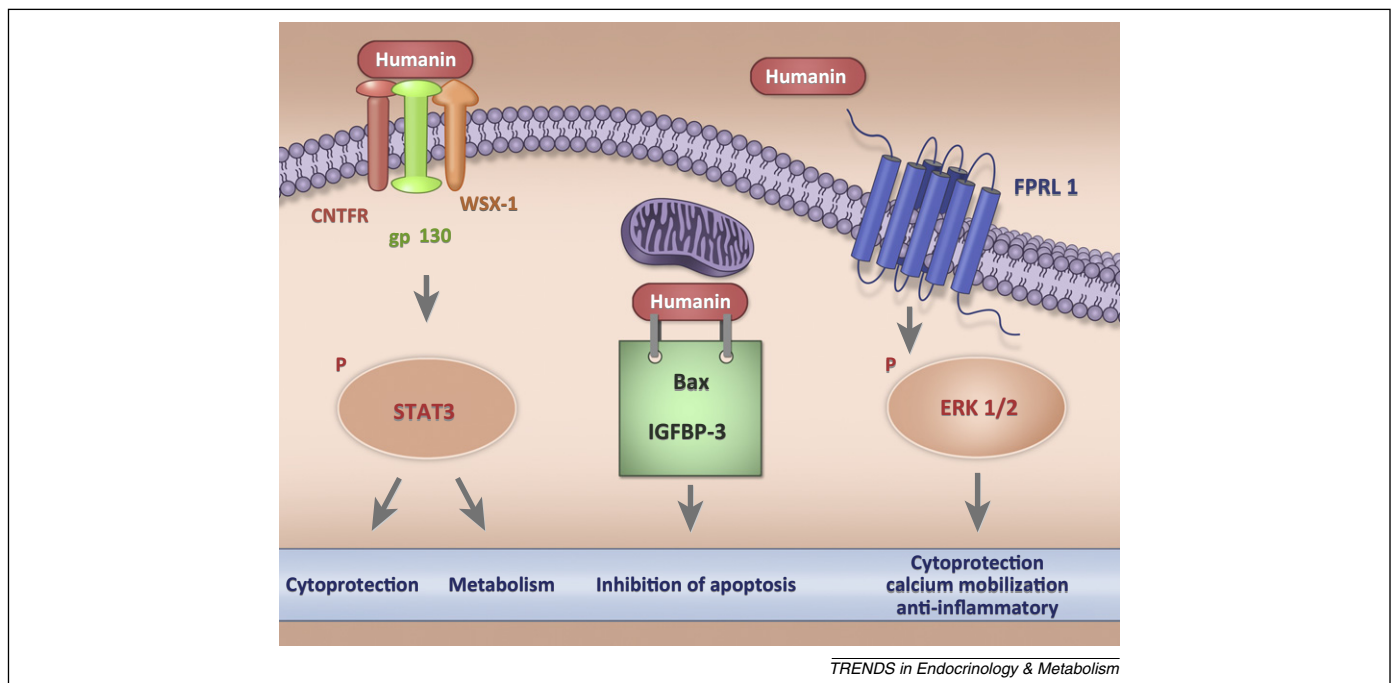


Figure 2. Cellular actions of humanin. Humanin has been shown to possess both intra- and extra-cellular modes of action. Within a cell, humanin interacts with pro-apoptotic proteins such as Bax and IGFBP-3, thereby preventing apoptosis. Extracellular humanin also regulates important cellular processes such as survival, metabolism, and inflammation via two types of cell-surface receptors; a trimeric receptor involving CNTFR/WSX-1/gp130, which relays signals through the STAT3 signaling pathway, and the formyl-peptide receptor-like-1 (FPRL1), which relays signals through the ERK 1/2 signaling cascade. P, phosphorylation.

matrix-protein fragments generated by proteolysis that are encoded in the nuclear genome and imported later into mitochondria. In a way, they are in a comparable class of retrograde signals with cytochrome *c* in that they are nucleus-encoded protein products released back to the cell from the mitochondria. In addition, mitochondrial DNA

(mtDNA) itself can act as a signal. A recent study demonstrated that inhibiting autophagy causes mitochondrial dysfunction, and subsequent mtDNA export to the cytosol, via increased mitochondrial membrane permeability transition (MPT; see [Glossary](#)), resulting in activation of the caspase-1 inflammasome [9].

Is humanin the first MDP?

Humanin is a polypeptide that is highly conserved across species. It was originally discovered in 2001 by screening a cDNA library created from the surviving fraction of an Alzheimer's disease (AD) patient brain [10,11]. Unbiased functional screening for clones that protect neuronal cells from cell death induced by amyloid precursor protein (APP) mutants, which are associated with early-onset familial AD (FAD) [10], identified multiple clones whose sequences were identical to a part of the mitochondrial 16S rRNA. Further functional and positional screening of the clones carrying various fragments of the 16S rRNA revealed a 75 bp open reading frame (ORF) sequence that yields a 24 amino acid peptide, which was designated humanin [10,12]. However, it is not clear whether humanin is translated in the mitochondrion or the cytoplasm. Mitochondria use a slightly different genetic code from that used in cytoplasmic translation to produce a distinct peptide from the same sequence. Humanin has been shown to be biologically effective when synthesized using both mitochondrial and cytoplasmic codes, and thus the translational site remains undetermined [11]. When the humanin ORF was cloned and expressed in neuronal cells it was again able to provide protection from several AD-related toxicities. The term humanin was coined by its discoverer, Professor Nishimoto, prior to his premature passing from cancer, to denote the potential of this molecule to restore the 'humanity' of patients with Alzheimer's disease.

A second group, using a yeast two-hybrid screen, also cloned humanin as a partner of the insulin-like growth factor-binding protein-3 (IGFBP-3), and demonstrated that humanin binds to IGFBP-3 with high affinity and specificity. IGFBP-3 regulates IGF bioactivity and also induces apoptosis and inhibits cell growth independently of IGFs, and humanin protects against IGFBP-3 induced apoptosis, discussed in detail below. However, humanin does not interfere with IGF-I/IGFBP-3 binding, but appears to bind to the heparin-binding domain (HBD) of IGFBP-3 [13]. Humanin has been also cloned by a third group as a binding partner of Bax, a central pro-apoptotic protein that translocates to the mitochondria to initiate a cell death signal. The humanin-Bax complex is retained in the cytoplasm and this interaction protects from apoptosis [11].

The humanin peptide sequence has been proposed to contain a 'pseudo-signal peptide', and when transfected into neuronal cells it is detected in the culture medium, indicating secretory capacity which is blocked by inhibition of the endoplasmic reticulum-Golgi transport processes [10]. In animals models and humans, humanin is found in tissues as well as in plasma. Cell-surface receptors for humanin have been proposed by several groups as noted below (Figure 2).

Mechanisms of action of humanin: receptors and signaling pathways

Humanin is secreted from cells and is found in circulation (plasma), as well as bound to cellular membranes [10,14], and its actions are thought to be mediated by specific receptors. To date, humanin has been shown to act 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) (Figure 2).

The first humanin receptor to be described was FPRL1, which has been linked to AD [15]. In neuroblasts, both the 42 amino acid form of the β -amyloid peptide ($A\beta_{42}$) and humanin were able to activate FPRL1, but only $A\beta_{42}$ was cytotoxic, and this cytotoxicity was reversed by humanin, suggesting that the neuroprotective effects of humanin might be accomplished by competitively binding to FPRL1 [15]. Although this study did not directly demonstrate that humanin binds to FPRL1, it did successfully show that humanin acts as an agonist for FPRL1 by inducing Ca^{2+} mobilization and rapid activation of ERK1/2 signaling, a commonly induced downstream pathway of G protein-coupled receptors, which might contribute to its cytoprotective aspects [15].

The second reported humanin receptor is the trimeric CNTFR/WSX-1/gp130 complex [16]. Hashimoto *et al.* previously showed that humanin induces STAT3 activation, which was required for its neuroprotective effects [16]. gp130 is a common element of receptors that belong to the interleukin-6 (IL-6) receptor family, and thus may be responsible for the signaling pathways mediated by Janus kinase (JAK)/STAT and ERK1/2 [16]. CNTF is a known IL-6 family cytokine. WSX-1 was found while testing gp130-coupling proteins that co-overexpressed with human gp130 [16]. Using *in vitro* pulldown assays, the study shows that humanin coprecipitated with the CNTFR α and the IL-27 subunit WSX-1, but not with IL-6 receptor α [16]. Furthermore, overexpression of CNTFR and/or WSX-1 results in enhanced humanin binding to neuronal cells, whereas siRNA-mediated knockdown of both or either component reduced binding. In addition, knockdown of CNTFR or WSX-1 abolishes humanin-dependent cytoprotection against AD-related insults [16]. Humanin also appears to promote the hetero-oligomerization of CNTFR, WSX-1, and gp130 [16].

Considering the diverse set of actions the humanin peptide elicits *in vitro*, it is possible that both types of receptors described above are important in mediating its various activities; however, specific experiments using genetic ablation of receptor subtypes need to be performed before more definitive roles of these pathways are identified.

Cytoprotective activities of humanin

The cytoprotective effects of humanin were first tested and confirmed *in vitro* against mutant forms of APP and presenilin 1 and 2 (PS1/2) which cause FAD [10]. F11 neuronal cells were challenged by transfecting APP, or PS1 or 2 cDNA, or by treating with $A\beta_{1-43}$ (25 μ M), and humanin was co-treated by either transfecting its cDNA or using synthesized peptides. Using trypan blue exclusion and WST-8 dyes, the study showed that humanin effectively protects neuronal cells from FAD-related toxicities.

As mentioned above, humanin also interacts with IGFBP-3 and Bax to antagonize their pro-apoptotic activity. Humanin also prevents Bax translocation, and activation, in response to the proapoptotic alkaloid staurosporine

Table 1. Cytoprotective activities of humanin

Type of stress/disease model	Description	Refs
Scopolamine	Acetylcholine muscarinic receptor antagonist-induced memory loss in mice	[47]
Dentatorubral-pallidoluysian atrophy (DRPLA)	An autosomal-dominant neurodegenerative disorder caused by expansion of CAG repeats (polyQ stretch) which form intracellular aggregates and confer neurotoxic activity	[48]
β -Amyloid (A β 25–35)	Cell death	[10,13,49]
	Behavioral deficits in mice	[50]
	Memory impairment in mice	[51]
Dexamethasone	Dexamethasone-induced apoptosis in rat Leydig cells	[52]
Familial amyotrophic lateral sclerosis (ALS)	Mice expressing a mutant superoxide dismutase (G93A-SOD1) (protection by Colivelin, a potent humanin derivative)	[53]
Intratesticular androgen deprivation	Intratesticular hormone deprivation-induced germ cell apoptosis (intratesticular HN administration)	[54]
Oxidized LDL	Oxidized LDL-induced oxidative stress in human aortic endothelial cells (HAEC)	[28]
Myocardial ischemia/reperfusion (IR) injury	Mouse model of mouse cardiac IR injury. 45 minutes of left coronary artery occlusion followed by 24 h reperfusion	[14]
Cerebral ischemia/reperfusion (IR) injury	Mouse model of cerebral IR injury. 75 minutes of cerebral artery occlusion followed by 24 h reperfusion	[55,56]
Oxygen–glucose deprivation (OGD)	Mouse primary cortical neurons under OGD for 60 minutes	[56]
Hypoxia (<i>in vitro</i>)	CoCl ₂ -induced apoptosis in transformed rat retinal ganglion cells	[57]
Triple transgenic AD mouse model (3 \times Tg-AD)	Memory deficit and β -amyloid accumulation in a triple transgenic AD mouse model (3 \times Tg-AD) harboring APPswe, tauP310L, and PS-1M146V	[58]
Double transgenic AD mouse model	Middle-aged double transgenic AD mouse model harboring APPswe/PS1dE9	[59]

Various studies have shown cytoprotective effects against a wide spectrum of stressors. Many of the stressors/models have oxidative stress as a common mechanism of action.

(STS) and subsequent release of cytochrome *c* into the cytoplasm [11]. Conversely, STS-treated Bax-null cells did show improved survival with humanin treatment. Humanin also binds to Bax-like BH3 proteins Bid and BimEL [17,18], and inhibits their proapoptotic activity. Endogenous humanin, produced by transfecting expression vectors, suppresses tBID- and BimEL-induced apoptosis *in vitro* [18,19]. These studies have broadened the scope of humanin activity by suggesting a crucial intracellular role in addition to the previously shown extracellular effects.

Following suit, several laboratories have tested the protective effect of humanin on various types of stress or disease states and have shown that humanin protects against them. Table 1 shows the types of stress or disease model used to test the protective effects of humanin. Although the specific molecular mechanism of cytotoxicity is unclear for some of these models, for example A β and 3 \times Tg-AD, many have oxidative stress as a common mechanistic denominator, suggesting a specific oxidative insult-related cytoprotective role of humanin.

Metabolic protection by humanin

Humanin appears to affect a number of metabolic disorders. Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) is a mitochondrial disorder strongly associated with A3243G mutant mtDNA, a location that is immediately downstream of the 16S rRNA locus. MELAS patients have defective ATP synthesis which is a major cause of tissue damage, especially those with high energy demands. Humanin restores ATP levels in lymphocytes from MELAS patients, preventing them from falling into energy deficiency [20]. Notably, humanin levels are considerably elevated in skeletal muscles of patients with

MELAS [21] and chronic progressive external ophthalmoplegia (CPEO), a mitochondrial disease associated with mtDNA abnormalities [22]. It is likely that the stress conferred by these pathological states could induce elevated levels of humanin.

Peripheral intravenous and central intracerebroventricular (ICV) infusion of humanin and its derivatives, F6AHN (a single amino acid substitution of alanine for phenylalanine at position 6; this substitution abrogates IGFBP-3 binding), HNG (a single amino acid substitution at position 14 (glycine for serine) that increases the biologic potency by \sim 1000-fold), and HNGF6A (contains both of these substitutions at positions 6 and 14, resulting in a potent non-IGFBP-3 binding peptide), significantly improve overall insulin sensitivity, mediated in part, by hypothalamic STAT3 activation in rats [23]. This effect has been suggested to be, in part, mediated by the CNTFR/WSX-1/gp130 trimeric humanin receptor. The enhanced insulin sensitivity following ICV HN administration in rats was also coupled with a significant reduction of hepatic glucose production (an indicator of hepatic insulin sensitivity) which, together with increased peripheral glucose uptake, raised the rate of glucose infusion in the euglycemic clamp study [23]. Furthermore, a single treatment with a highly potent humanin analog (HNGF6A) normalizes blood glucose levels in Zucker diabetic fatty rats, a model of type 2 diabetes.

In addition, humanin improves glucose tolerance and onset of diabetes in NOD mice, a model of type 1 diabetes mellitus (T1DM) that involves progressive accumulation of lymphocytes within the pancreatic islets of Langerhans [24,25]. In NOD mouse pancreata, humanin treatment was shown to dramatically suppress inflammation and lymphocyte infiltration. Furthermore, humanin treatment

decreases serum starvation- and cytokine-induced apoptosis in the pancreatic β -cell line NIT-1 [24], suggesting that it is islet protective and thus might be considered for a new therapeutic target for T1DM, a chronic autoimmune disease with destruction of the β -cells in the islets of Langerhans [26]. In addition, cytokines including IL-1 β , tumor necrosis factor (TNF) α , and interferon (IFN) γ released by T cells and activated macrophages, have a role in β -cell destruction; humanin was able to protect NIT-1 cells from these cytokine-induced apoptosis in an ERK- and STAT3-dependent manner [24].

Humanin modulates oxidative stress and apoptosis in the developing plaque to protect endothelial function and prevent atherosclerosis. Lerman and colleagues treated ApoE-deficient mice fed a high cholesterol diet with the humanin analog HNGF6A for 16 weeks and showed a dramatic decrease of the atherosclerotic plaque size, preserved endothelial dysfunction, and improved expression of endothelial NO synthase (eNOS) in aorta [27]. This study suggests that anti-oxidative stress, antiapoptosis, and preservation of eNOS are mechanisms partially responsible for the protection against atherosclerosis by humanin.

Interestingly, humanin levels appear to decline with age [23,28], suggesting that a relative deficiency of humanin may be involved in metabolic diseases during aging. In one study, older mice had significantly reduced levels of circulating humanin levels (2 vs 13 months of age), and rats had decreased levels of hypothalamic and skeletal muscle humanin (3 vs 24 months). The study also reported that circulating levels of humanin also gradually decline with age in humans (45–65 vs 66–80 vs 81–100 years of age) [23]. In another study, plasma humanin levels also significantly decreased with age in healthy volunteers (39 ± 4 vs 60 ± 6 years) [28].

The emerging concept of MDPs – evidence from model organisms

Humanin may be the first small peptide of its kind that represents a putative set of MDPs, a novel concept that can radically change the traditional thinking about retrograde mitochondrial signaling as well as mitochondrial gene expression. However, polyadenylated mitochondrial RNA transcripts have been previously cloned from gene libraries. In the early 1980s, a cDNA library constructed from interferon-induced human myeloblast cells was used to find interferon-induced-specific sequences [29]; the majority of the sequences with strong induction mapped to the mitochondrial 12S and 16S rRNA regions.

A recent report supports the concept of specific mitochondrial-derived signaling with endocrine properties. In the nematode *C. elegans*, a stress-induced tissue-specific signal originating from mitochondria can act on distant organs to regulate lifespan. Neuron-specific disruption of mitochondria induces the mitochondrial unfolded-protein response (UPR) in worm intestine, and leads to an extension of lifespan, most likely mediated by an unidentified circulating signal(s) that allows the mitochondria of neuronal and intestinal cells to communicate stress conditions [30,31]. These studies strongly support the novel concept of specific and active mitochondria-initiated signals that play

significant roles in cellular stress response and longevity. However, these studies did not provide the specific identity or function of such signals. Although direct evidence has yet to be shown, the involvement of MDPs should be considered because, as mentioned above, currently identified stress-induced mitochondrial retrograde signals are cell-autonomous (e.g., fragmented mitochondrial proteins [8] or mtDNA [9]), whereas humanin is also an endocrine factor that can act non-autonomously.

The mitochondrial transcriptome and its implications

Compared to the human nuclear genome, mitochondria have a modestly sized circular genome of $\sim 16\,570$ bp, which includes only 13 protein-coding genes, all encoding structural components of the electron transport chain system [32]. Nevertheless, despite its small size, our understanding of its gene regulation and protein expression is very crude.

mtDNA replication and transcription starts are regulated by nucleus-encoded proteins. The entire genome is thought to be transcribed as a single polycistronic precursor that is processed into individual genes by excising the strategically positioned 22 tRNAs (tRNA punctuation model), giving rise to two rRNAs and 13 mRNAs [32]. In addition, owing to cutting-edge technology such as near-exhaustive deep sequencing that allows single-base resolution, a comprehensive map of the human mitochondrial transcriptome is available that gives access to previously unseen short RNA species [32].

The human mitochondrion has two promoters in the heavy strand (major and minor) in close proximity, and one in the light strand, thereby giving rise to three different single polycistronic transcripts; the heavy major promoter is responsible for 80% of all mtRNA transcripts. Nevertheless, although the entire genome is thought to be transcribed as a single transcript, the abundance of individual rRNA, tRNA, and mRNA transcripts varies greatly, and the rRNAs are the most abundant, indicating an unexplored class of post-transcriptional processing in the mitochondria [32]. Importantly, many of the mRNA species identified from the mitochondria are discrete smaller-length ones that do not map to the traditional mitochondrial protein-coding genes. In particular, multiple such mRNAs are observed from the 16S rRNA region including the site of the humanin ORF. Considering its bacterial ancestry, it is not entirely surprising to find that such a compact genome evolved with a polycistronic strategy that includes ‘genes-with-genes’ [32]. Furthermore, recent evidence shows that polycistronic gene arrangements are not exclusive to prokaryotes but also used by eukaryotes [33,34].

Using parallel analysis of RNA ends (PARE) to map the transcript cleavage sites, a plethora of expected and unexpected cleavage sites have been discovered for the mitochondrion [32]. The majority of tRNAs and mRNA had distinct dominant cleavage site at the 5' termini, but intragenic cleavage sites are especially abundant in rRNAs.

The latter stages of precursor modification include 3' polyadenylation of mRNAs, with the exception of the mitochondrial ND6 gene, and rRNAs, in agreement with

previous reports [32,35]. Although the precise role of rRNA polyadenylation is still unknown, the humanin cDNA clone was found to be polyadenylated. As mentioned above, the mitochondrion evolved to maintain a polycistronic transcript which requires a sophisticated regulatory machinery to process each gene separately. Notably, there is compelling evidence from the emerging field of small peptides showing biologically active peptides of 11–32 amino acids in length which are encoded by small open reading frames (sORFs) from a polycistronic mRNA [33,36,37]. In addition, mitochondrial rRNAs are transported and found in the cytoplasm with significant biological roles in, for instance, *Drosophila* germ-cell establishment [38], and the 16S rRNA was found localized in the nucleus of human spermatogenic cells [39]. It is possible that the polyadenylated humanin transcript is exported to the cytoplasm where it can be translated.

Curiously, the mitochondrial transcriptome includes small regulatory RNAs. Furthermore, multiple transcription binding sites determined by high-throughput *in vivo* footprinting assay and higher-order organization of the mitochondrial genome were shown [32]. Shuttling of RNA to and from the mitochondria is evident and may involve mRNA binding proteins such as PNPase [40]. Current understanding is insufficient to explain the more recent observations and is in urgent need of revision and amendments.

The concept of NUMTs and evidence for the mitochondrial origin of humanin

The incorporation of the ancient α -proteobacterium has immensely accelerated the evolutionary course of metazoan cells. During the prolonged transition from endosymbiotic residence to organelle status in host cells, mitochondria are thought to have transferred most of its genome to the host nucleus, leaving chromosomal ‘Doppelgangers’ through the process of mtDNA transfer to the nucleus or nuclear insertions of mitochondrial origin (NUMTs) [41,42].

NUMTs come in various sizes from all parts of the mtDNA, with various degrees of homology with the original sequences, and the ‘partial ghosts’ of entire mtDNA can be found in the nuclear genome, although in most cases with substantial sequence degeneration [43]. In humans, there are an estimated 755 insertions dispersed throughout the nuclear genome, with chromosome two having the most insertions [42] and chromosome 18 having the least [42]. Most NUMTs are small insertions of <500 bp and only 12.85% are >1500 bp [42]. The percentage identity is inversely correlated with size, and the mean percentage between NUMTs and mtDNA is 79.2%, with a range of 63.5% to 100% identity [43].

To date, the mitochondrial origin of humanin is still debated. NUMTs that reflect humanin sequences are found in chromosomes 1–13 in varying degrees of homology, but none of the peptide sequences deduced from NUMTs are fully identical to the original cDNA clone from the AD patient brain cloned by Nishimoto and colleagues, or the HeLa cell library cloned by Ikonen, both of which were 100% identical to the mitochondrial sequence [44].

Furthermore, northern blot analysis using the humanin ORF (antisense) as the probe showed hybridization to total

RNA, from HeLa cells, which was identical in size to mitochondrial 16S rRNA, whereas total RNA from ρ 0 HeLa cells, which lack mtDNA, did not show any significant hybridization [45]. Nonetheless, synthetic peptides corresponding to a NUMT origin that have 90% amino acid homology to the mitochondrial humanin retain some (incomplete) biological activity [44]. On the other hand, formylated humanin has enhanced activity compared to humanin by increasing the affinity to FPRL1, a humanin receptor, supporting a mitochondrial origin because N-termini formylation is a hallmark of mitochondrion-encoded proteins [46]. Thus, strong circumstantial data supports a mitochondrial origin for humanin, but a NUMT-derived humanin has not been ruled out yet.

Concluding remarks

Humanin may be a harbinger of additional MDPs because numerous putative ORFs are dispersed throughout the mitochondrial genome. Humanin is the first described MDP shown to be a neuroprotective peptide, and has rapidly expanded its protective repertoire to various types of stress and disease models. It should be noted that many of these models have oxidative stress as a common denominator, begging the question whether humanin is a more general cytoprotective factor against oxidative stress. Interestingly, humanin levels decline with age, which is a major risk factor for many of the diseases in which humanin is protective, including AD, atherosclerosis, myocardial and cerebral ischemia, and type 2 diabetes, all of which involve an oxidative stress component.

The emerging biology of MDPs represents the foreground of novel mitochondrial regulation of cellular activity. Deciphering the details of these processes, and identifying further related genes and peptides, holds therapeutic and diagnostic potential in a wide spectrum of human diseases.

References

- Gray, M.W. (2012) Mitochondrial evolution. *Cold Spring Harb. Perspect. Biol.* 4, a011403
- Wallace, D.C. (2011) Bioenergetic origins of complexity and disease. *Cold Spring Harb. Symp. Quant. Biol.* 76, 1–16
- Nunnari, J. and Suomalainen, A. (2012) Mitochondria: in sickness and in health. *Cell* 148, 1145–1159
- Wallace, D.C. (2012) Mitochondria and cancer. *Nat. Rev. Cancer* 12, 685–698
- Verdin, E. *et al.* (2010) Sirtuin regulation of mitochondria: energy production, apoptosis, and signaling. *Trends Biochem. Sci.* 35, 669–675
- Ganta, V.C. and Alexander, J.S. (2009) Focus on carbon monoxide: a modulator of neutrophil oxidants and elastase spatial localization? *Am. J. Physiol. Heart Circ. Physiol.* 297, H902–H904
- Ichikawa, Y. *et al.* (2012) Disruption of ATP-binding cassette B8 in mice leads to cardiomyopathy through a decrease in mitochondrial iron export. *Proc. Natl. Acad. Sci. U.S.A.* 109, 4152–4157
- Haynes, C.M. *et al.* (2010) The matrix peptide exporter HAF-1 signals a mitochondrial UPR by activating the transcription factor ZC376.7 in *C. elegans*. *Mol. Cell* 37, 529–540
- Nakahira, K. *et al.* (2011) Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat. Immunol.* 12, 222–230
- Hashimoto, Y. *et al.* (2001) A rescue factor abolishing neuronal cell death by a wide spectrum of familial Alzheimer’s disease genes and Abeta. *Proc. Natl. Acad. Sci. U.S.A.* 98, 6336–6341
- Guo, B. *et al.* (2003) Humanin peptide suppresses apoptosis by interfering with Bax activation. *Nature* 423, 456–461

- 12 Hashimoto, Y. *et al.* (2001) Mechanisms of neuroprotection by a novel rescue factor humanin from Swedish mutant amyloid precursor protein. *Biochem. Biophys. Res. Commun.* 283, 460–468
- 13 Ikonen, M. *et al.* (2003) Interaction between the Alzheimer's survival peptide humanin and insulin-like growth factor-binding protein 3 regulates cell survival and apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13042–13047
- 14 Muzumdar, R.H. *et al.* (2010) Acute humanin therapy attenuates myocardial ischemia and reperfusion injury in mice. *Arterioscler. Thromb. Vasc. Biol.* 30, 1940–1948
- 15 Ying, G. *et al.* (2004) Humanin, a newly identified neuroprotective factor, uses the G protein-coupled formylpeptide receptor-like-1 as a functional receptor. *J. Immunol.* 172, 7078–7085
- 16 Hashimoto, Y. *et al.* (2009) Humanin inhibits neuronal cell death by interacting with a cytokine receptor complex or complexes involving CNTF receptor alpha/WSX-1/gp130. *Mol. Biol. Cell* 20, 2864–2873
- 17 Choi, J. *et al.* (2007) Mapping the specific cytoprotective interaction of humanin with the pro-apoptotic protein bid. *Chem. Biol. Drug Des.* 70, 383–392
- 18 Luciano, F. *et al.* (2005) Cytoprotective peptide humanin binds and inhibits proapoptotic Bcl-2/Bax family protein BimEL. *J. Biol. Chem.* 280, 15825–15835
- 19 Zhai, D. *et al.* (2005) Humanin binds and nullifies Bid activity by blocking its activation of Bax and Bak. *J. Biol. Chem.* 280, 15815–15824
- 20 Kariya, S. *et al.* (2005) Effect of humanin on decreased ATP levels of human lymphocytes harboring A3243G mutant mitochondrial DNA. *Neuropeptides* 39, 97–101
- 21 Kariya, S. *et al.* (2005) Humanin detected in skeletal muscles of MELAS patients: a possible new therapeutic agent. *Acta Neuropathol.* 109, 367–372
- 22 Kin, T. *et al.* (2006) Humanin expression in skeletal muscles of patients with chronic progressive external ophthalmoplegia. *J. Hum. Genet.* 51, 555–558
- 23 Muzumdar, R.H. *et al.* (2009) Humanin: a novel central regulator of peripheral insulin action. *PLoS ONE* 4, e6334
- 24 Hoang, P.T. *et al.* (2010) The neurosurvival factor Humanin inhibits beta-cell apoptosis via signal transducer and activator of transcription 3 activation and delays and ameliorates diabetes in nonobese diabetic mice. *Metabolism* 59, 343–349
- 25 Wicker, L.S. *et al.* (1995) Genetic control of autoimmune diabetes in the NOD mouse. *Annu. Rev. Immunol.* 13, 179–200
- 26 Bluestone, J.A. *et al.* (2010) Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature* 464, 1293–1300
- 27 Oh, Y.K. *et al.* (2011) Humanin preserves endothelial function and prevents atherosclerotic plaque progression in hypercholesterolemic ApoE deficient mice. *Atherosclerosis* 219, 65–73
- 28 Bachar, A.R. *et al.* (2010) Humanin is expressed in human vascular walls and has a cytoprotective effect against oxidized LDL-induced oxidative stress. *Cardiovasc. Res.* 88, 360–366
- 29 Maeda, S. *et al.* (1980) Construction and identification of bacterial plasmids containing nucleotide sequence for human leukocyte interferon. *Proc. Natl. Acad. Sci. U.S.A.* 77, 7010–7013
- 30 Durieux, J. *et al.* (2011) The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell* 144, 79–91
- 31 Woo, D.K. and Shadel, G.S. (2011) Mitochondrial stress signals revise an old aging theory. *Cell* 144, 11–12
- 32 Mercer, T.R. *et al.* (2011) The human mitochondrial transcriptome. *Cell* 146, 645–658
- 33 Savard, J. *et al.* (2006) A segmentation gene in tribolium produces a polycistronic mRNA that codes for multiple conserved peptides. *Cell* 126, 559–569
- 34 Tautz, D. (2009) Polycistronic peptide coding genes in eukaryotes – how widespread are they? *Brief. Funct. Genomics Proteomics* 8, 68–74
- 35 Baserga, S.J. *et al.* (1985) Polyadenylation of a human mitochondrial ribosomal RNA transcript detected by molecular cloning. *Gene* 35, 305–312
- 36 Kondo, T. *et al.* (2010) Small peptides switch the transcriptional activity of Shavenbaby during *Drosophila* embryogenesis. *Science* 329, 336–339
- 37 Galindo, M.I. *et al.* (2007) Peptides encoded by short ORFs control development and define a new eukaryotic gene family. *PLoS Biol.* 5, e106
- 38 Amikura, R. *et al.* (2001) Presence of mitochondria-type ribosomes outside mitochondria in germ plasm of *Drosophila* embryos. *Proc. Natl. Acad. Sci. U.S.A.* 98, 9133–9138
- 39 Villegas, J. *et al.* (2002) Localization of the 16S mitochondrial rRNA in the nucleus of mammalian spermatogenic cells. *Mol. Hum. Reprod.* 8, 977–983
- 40 Wang, G. *et al.* (2010) PNPASE regulates RNA import into mitochondria. *Cell* 142, 456–467
- 41 Bensasson, D. *et al.* (2001) Mitochondrial pseudogenes: evolution's misplaced witnesses. *Trends Ecol. Evol.* 16, 314–321
- 42 Ramos, A. *et al.* (2011) Nuclear insertions of mitochondrial origin: Database updating and usefulness in cancer studies. *Mitochondrion* 11, 946–953
- 43 Mourier, T. *et al.* (2001) The Human Genome Project reveals a continuous transfer of large mitochondrial fragments to the nucleus. *Mol. Biol. Evol.* 18, 1833–1837
- 44 Bodzioch, M. *et al.* (2009) Evidence for potential functionality of nuclearly-encoded humanin isoforms. *Genomics* 94, 247–256
- 45 Tajima, H. *et al.* (2002) Evidence for in vivo production of Humanin peptide, a neuroprotective factor against Alzheimer's disease-related insults. *Neurosci. Lett.* 324, 227–231
- 46 Harada, M. *et al.* (2004) N-Formylated humanin activates both formyl peptide receptor-like 1 and 2. *Biochem. Biophys. Res. Commun.* 324, 255–261
- 47 Mamiya, T. and Ukai, M. (2001) [Gly(14)]-Humanin improved the learning and memory impairment induced by scopolamine in vivo. *Br. J. Pharmacol.* 134, 1597–1599
- 48 Kariya, S. *et al.* (2005) Humanin attenuates apoptosis induced by DRPLA proteins with expanded polyglutamine stretches. *J. Mol. Neurosci.* 25, 165–169
- 49 Jin, H. *et al.* (2010) Protective effects of [Gly14]-Humanin on beta-amyloid-induced PC12 cell death by preventing mitochondrial dysfunction. *Neurochem. Int.* 56, 417–423
- 50 Miao, J. *et al.* (2008) S14G-Humanin ameliorates Abeta25-35-induced behavioral deficits by reducing neuroinflammatory responses and apoptosis in mice. *Neuropeptides* 42, 557–567
- 51 Tajima, H. *et al.* (2005) A humanin derivative, S14G-HN, prevents amyloid-beta-induced memory impairment in mice. *J. Neurosci. Res.* 79, 714–723
- 52 Colon, E. *et al.* (2006) Anti-apoptotic factor humanin is expressed in the testis and prevents cell-death in leydig cells during the first wave of spermatogenesis. *J. Cell. Physiol.* 208, 373–385
- 53 Chiba, T. *et al.* (2006) Colivelin prolongs survival of an ALS model mouse. *Biochem. Biophys. Res. Commun.* 343, 793–798
- 54 Lue, Y. *et al.* (2010) Opposing roles of insulin-like growth factor binding protein 3 and humanin in the regulation of testicular germ cell apoptosis. *Endocrinology* 151, 350–357
- 55 Xu, X. *et al.* (2006) Humanin is a novel neuroprotective agent against stroke. *Stroke* 37, 2613–2619
- 56 Xu, X. *et al.* (2008) Neuroprotective effect of humanin on cerebral ischemia/reperfusion injury is mediated by a PI3K/Akt pathway. *Brain Res.* 1227, 12–18
- 57 Men, J. *et al.* (2012) An AD-related neuroprotector rescues transformed rat retinal ganglion cells from CoCl₂-induced apoptosis. *J. Mol. Neurosci.* 47, 144–149
- 58 Niikura, T. *et al.* (2011) A humanin derivative reduces amyloid beta accumulation and ameliorates memory deficit in triple transgenic mice. *PLoS ONE* 6, e16259
- 59 Zhang, W. *et al.* (2012) S14G-humanin improves cognitive deficits and reduces amyloid pathology in the middle-aged APPswe/PS1dE9 mice. *Pharmacol. Biochem. Behav.* 100, 361–369