Therapeutic potential of melatonin related to its role as an autophagy regulator: A review

Running title: Melatonin as an autophagy regulator

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ABSTRACT

There are several pathologies, syndromes and physiological processes in which autophagy is involved. This process of self-digestion that cells trigger as a survival mechanism is complex and tightly regulated, according to the homeostatic conditions of the organ. However, in all cases, its relationship with oxidative stress alterations is evident, following a pathway that suggests endoplasmic reticulum stress and/or mitochondrial changes. There is accumulating evidence of the beneficial role that melatonin has in the regulation and restoration of damaged autophagic processes. In this review, we focus on major physiological changes such as aging and essential pathologies including cancer, neurodegenerative diseases, viral infections and obesity, and document the essential role of melatonin in the regulation of autophagy in each of these different situations.

Key words: melatonin, autophagy, aging, neurodegenerative diseases, cancer, obesity, virus infection, endoplasmic reticulum stress, mitochondria.

INTRODUCTION

Autophagy, as a mechanism genetically well preserved, is an essential cellular process in which eukaryotic cells totally or partially self-digest cytoplasmic components in order to counteract toxic or damaged products and, initially, to maintain cellular homeostasis.[1] Autophagy induction leads to a series of coordinated processes that begin with the phagophore, a membrane structure that progressively expands, engulfing cytoplasmic portions or damaged cargos (Fig. 1). The phagophore evolves into an autophagosome, a double membrane structure that is the morphological hallmark of autophagy.[2] This autophagosome binds to a lysosome, losing one of its membranes and delivering its content for degradation.[3] Three different types of lysosome-based autophagic pathways have been described: a) microautophagy, a constitutive process in charge of degrading deteriorated elements of the cell, which is maintained throughout the life of cells and which delays senescence; b)chaperone-mediated autophagy (CMA), which selectively degrades cellular proteins with KFERQ-like motif via unique machinery and c) macroautophagy, the most common autophagy and usually referred to simply as autophagy.[4]

Implications of the importance of autophagy in alterations of cellular processes and pathologies are becoming more evident. Cell cleaning processes induced by toxic or harmful situations appear to be essential, and in many cases of cell survival or death are functionally solved by autophagic processes. Likewise, protein homeostasis or proteostasis is usually related to adequate autophagy development, allowing a balance between protein synthesis and degradation.[5]

Many factors trigger autophagy, which involve multiple steps and types; this makes it difficult to understand the role of autophagy in each process. Thus, to clarify the context or circumstances where autophagy comes into play and is essential to

identify the consequences of its alteration. Thus, we consider that any study of the relationship of these mechanisms of cell cleaning and survival must occur, in any pathology, by placing it in its corresponding environment.

The dual role of autophagy in cell survival or death depends on the severity of cellular damage[4] as shown by its trigger inducers which includes several essential cell components: mitochondria, endoplasmic reticulum, peroxisomes, lysosomes. Of these autophagy inducers, mitochondria due to the loss of energy generated in the cell and endoplasmic reticulum through the stress of the reticulum, are those that cause more widespread damage. Endoplasmic reticulum stress induces the production of a large amount of misfolded proteins that trigger a known response in the cell: unfolded protein response (UPR) that follows three routes whose receptor proteins are: inositol-requiring kinase 1 (IRE-1), activating transcription factor 6 (ATF6) and protein kinase RNA-like endoplasmic reticulum kinase (PERK) (Fig. 2). Since common direct consequence of these important cell alterations is the oxidative stress, quantity of free radical damageexplains why autophagy changes in response to slight variations in oxidative stress.[6,7] There are numerous recent articles that show the relationship of oxidative stress to autophagy in multiple pathologies. Yet, it is surprising how this known association has not served as a trigger for extensive research based on the roles of antioxidants in the regulation of autophagy.

The indolamine, melatonin,which was firstly discovered to be a skin lightening agent in frogs and fish, is mostly considered a circadian rhythm regulator produced by pineal gland. It is also synthesized in numerous (perhaps all) tissues in both animal and plant species, where it functions as a local redox regulator.[4,8,9,10] Briefly, the high effectiveness of melatonin as an antioxidant is based on: its high efficiency as a radical scavenger, its amphiphilic nature which allows it to pass easily all bio-barriers and its

capacity to enter mitochondria were many oxygen and nitrogen toxic species are generated.[11-14] Melatonin also protects essential cell elements such as nucleus,[11] mitochondria[15] and endoplasmic reticulum.[16] Alterations in melatonin have been associated with multiple pathologies ranging from mood disorders[17] to retinopathies.[18] However, its role in the regulation of autophagic processes has not been extensively investigated in much pathology.

It is the intent of this review, not only to show the intimate relationship that exists between melatonin and autophagy in various pathologies due to the common association with that oxidative stress, but also, and perhaps preeminently, to identify some of the many pathologies where melatonin and the autophagic processes remain unexplored. The lack of information in this area is surprising considering the negative effects caused by the increase in free radical production[19-21] and clearly established relationship between oxidative stress and autophagy in a variety of diseases.[22, 23]

Given this information, this review not only shows the beneficial role of melatonin in important pathologies that present autophagic alterations, but also encourages the scientific community to carry out additional studies as to their specific interactions considering new fields of application (e.g., viral infections, obesity).

AGING

Aging is a physiological and multifactorial process that provokes gradual cellular deterioration in most living organisms (Fig. 3). This process isnot only associated with compromised lifespan, but it also has a marked effect on healthspan. In fact, aging results in an increased susceptibility to a variety of neurodegenerative diseases, eating disorders, viral infections and cancers.[24-26] Specific increases of reactive oxygen species (ROS) are considered as a key regulator of aging and tissue decline, due

to their role in directing the cell towards life or death.[27, 28] The importance of melatonin in aging and in age-related diseases is underlined by the reduced production of this potent antioxidant in the pineal gland,[29,30] as well as, the reduction of MT1 and MT2 melatonin receptor expression in extrapineal tissues during physiological aging.[31] A defect in antioxidant defense results in a failed responsiveness to protect against oxidative stress, thereby reducing longevity.[32] Consequently, melatonin has now become an agent of interest in anti-aging medicine. Studies completed to date suggest that the exogenous administration of melatonin may have a role in extending normal longevity as shown in several model organisms.[33-36] However, further studies on the use of melatonin in clinical trials in the elderly are needed to support this conclusion in humans. Understanding the underlying molecular mechanisms of aging processes has become critical for identifying melatonin's role in anti-aging and developing health-care strategies.[37]

Recently, a large number of studies have focused on the role of mitochondria and the basal energy availability as critical factors for the maintenance of tissue homeostasis and the senescence process. Mitochondria contribute to cellular function regulation including cell proliferation, the cell cycle and apoptotic processes.[38] A decline in mitochondrial function is associated with aging and correlates with the production of excessive amounts of ROS from the electron transport chain, which, in turn, damage phospholipids, proteins and especially mitochondrial DNA. This causes further mitochondrial dysfunction and the consequent age-related physiological decline.[39-42] As a result, many tissues from aged individuals exhibit diminished mitochondrial respiratory enzyme activities and, therefore, also lower energy production leading to decreased mitochondrial membrane potential.[43-45] It is noteworthy that mitochondrial dysfunction is of paramount importance in post-mitotic tissues, such as

skeletal muscle and brain. This reduction and function is associated with damaged mitochondria and contributes to the onset of aging.[46-49] With impaired mitochondrial function, the maintenance of cellular homeostasis is strongly dependent on the appropriate function of quality control mechanisms.

The task of managing tissue damage is largely under the control of autophagy, which is regulated through the AMPK/mTOR pathway. Recent work has pointed to a further role of AMPK/mTOR as a master energy sensor that contributes to metabolic adaptation and enables cells to survive unfavorable conditions.[50,51] Thus, autophagy is not only activated in response to the accumulation of cellular damage, but in fact, one of its strongest stimuli is nutrient deprivation which allows cells to re-utilize their constituents for energy.[52] Given that the time-dependent accumulation of cellular damage and the lack of energy production are considered as two hallmarks of the aging phenotype in different organisms,[53] the lifespan extension is strongly dependent on the regulation of the autophagy response. Many studies have demonstrated that the autophagic capacity observed in different tissues decreases during the course of aging and has been proposed as a sign of the aged cell.[54-57] In fact, it has been attributed this age-related decline in autophagy as a cause for accumulating altered molecules resulting from damaged and dead cells, which triggers inflammation response. The term of "garb-aging" has been coined to define this process.[58] In addition, adverse conditions such as increased fat mass, influence age-related decline and mitochondrial dysfunction that aggravate the impaired autophagy response during aging.[59,60] A study in Atg7-deficient mice provides support for the important role of autophagy in the quality control of organelles in quiescent cells, in particular mitochondria, due a deficit turnover that results in cellular dysfunction.[61] This explains why model organisms with mitochondrial

dysfunction and an impaired autophagy response exhibit accelerated aging which contributes to increased age-related pathologies.[62]

Recent research has also revealed a strong interplay between perturbations in the cellular redox state and acute inhibition of autophagy, due to the direct oxidation of Atg3 and Atg7 that compromises autophagosome maturation.[63] There is also a higher ROS-mitochondria co-localization in geriatric satellite cells which correlates with impaired mitophagic and autophagic flux.[64] This was observed when these workers examined the re-establishment of the autophagy capacity by overexpressing Atg7, convincingly demonstrating the senescence reversion and the intrinsic regenerative functions rescue the formation of new muscle fibers. In addition, a later work demonstrated that a targeted Phe121 Ala mutation in Beclin 1 disrupts the Beclin1-BCL2 binding that results in increased basal levels of autophagy in several tissues.[65] Notably, the reversion of theBeclin1-BCL2 autophagy regulatory complex formation prevents premature aging, extends longevity, improves healthspanand also rescues organisms from early death, these studies in model organisms show that autophagy regulation can extend lifespan and they provide evidence that the aging process, a major biomedical challenge, can be modulated.

Numerous publications have confirmed the protective effects of melatonin against oxidative stress; however, there are a limited number of studies focused on the impact of melatonin on the autophagy pathway during aging. The connection between the deregulation of oxidant production that compromises the cellular reducing system and its inhibitory effect on autophagy capacity in tissue aging,[63] documents a role for melatonin as a possible regulator of autophagy through its redox-mediated actions. Pioneering studies haves howed the effectiveness of melatonin against oxidative damage and apoptosis response in the brain of aged mice, without a clear modification

of macroautophagy.[66,67] However, recent findings *in vitro* and *in vivo* have reported that melatonin regulates autophagy processes due to its role as a metabolic regulator and mitochondrial protector. Indeed, these studies gained particular attention driven by the finding that melatonin accumulates in mitochondria, supporting the presence of regulatory mechanisms in these cellular organelles.[68] One of the most beneficial effects of melatonin appears to be the protection of the electron transport chain and mitochondrial DNA from oxidative damage thereby normalizing the redox status, which in turn increases ATP production and prevents the decay of the membrane potential.[69-72]

The regulation of mitochondrial membrane fluidity by melatonin treatment also seems to contribute to the maintenance of structural pathways and slow down the aging process by the modulation of age-related autophagy-lysosomal alterations.[73] The most recent studies have focused on the connection between SIRT1, a NAD-dependent deacetylase, and the circadian clock.[74] SIRT1 is directly linked to the regulation of mitochondrial metabolism and aging.[75] Moreover, SIRT1 is also a positive regulator of autophagy and mitochondrial function in embryonic stem cells; its effects are mediated in part via PI3K/Beclin1 and mTOR pathways.[76] Further studies have demonstrated how melatonin is able to reverse H_2O_2-induced senescence in a human-derived cell line by triggering enhanced autophagy response through a SIRT1-catalyzed deacetylation.[77] Thus, melatonin may be considered a protective compound that can modulate autophagy and attenuate aging conditions. Another study indicated a novel role of melatonin as a protector against autophagic cell death by mediating the dissociation of the Beclin1-BLC2 complex.[78] Overall, this report together with the finding that disruption of Beclin1-BCL2 autophagy regulatory complex extends lifespan, previously described,[65] suggests the possibility of the utilization of melatonin as therapeutic strategy in the anti-aging processes.[37]

In summary, the regulatory role of melatonin on autophagic mechanism requires future attention, particularly when considering its relevance for extending longevity and improving healthspan. This is especially important in the elderly who achieve an age-related melatonin deficient state. Therefore, melatonin administration as a supplement to normalize normal endogenous levels may delay age-related degeneration (Fig. 3).

NEURODEGENERATIVE DISEASES

Neurodegeneration is defined as a process where anatomical subsets of neurons exhibit a functional and structural loss.[79] Alzheimer´s (AD), Parkinson´s (PD) and Huntington's diseases (HT) are recognized as the most common age-related neurogenerative diseases, which share pathological markers that include an important neuronal loss derived from mitochondrial dysfunction, exacerbated oxidative stress, neuroinflammation and toxic accumulation of proteins and/or aggregates.[66,67,79,80] Notably, several other neuropathological situations lead to neurodegeneration, including traumatic brain injury, hypoxia, ischemia and reperfusion processes, cerebral hemorrhage, neurotoxin exposure and brain infection.[81-90] Mitochondrial dysfunction is one of the earliest and most relevant destructive events in neurodegenerative processes that leads to oxidative stress, impairments of mitochondrial dynamics and its genomic integrity as well as cell death activation.[80] In addition to elimination of toxic protein aggregates, disposal of non-functional mitochondria seems to be of paramount importance in the cells that fight against neurodegeneration.

Autophagy is a stress response cell mechanism with a key role in the physiology of the CNS. It allows the maintenance of a proper neuronal homeostasis and function. In fact, autophagy seems to play a key role in synaptic growth and plasticity required for learning and memory such that cognitive function is intimately associated with

autophagy activity in the brain; this especially relates to the aging and neurodegenerative conditions, where autophagy is compromised to a greater or lesser extent.[92,93] However, uncontrolled and exacerbated autophagy may lead to type II programmed cell death and significantly contribute to neurodegenerative pathology.[79,80] An instance of this dual role of autophagy has been extensively demonstrated in AD since while autophagy inducers provide effective therapeutic strategies by degrading aggregates in the early stages of AD, autophagy induction increases disease severity during the late stages of the disease by accelerating amyloid β-peptides production.[93]

Melatonin is as neuroprotective agent against diseases such as AD, PD and HD due to its high antioxidant potential[66,67,80,94,95]. Beneficial effects of melatonin in several other neurotoxicitic conditions have been also demonstrated.[8,81-84,87,88,95] The main benefits of melatonin in models of neurodegeneration are linked to mitochondria, since melatonin is able to counteract oxidative stress, prevent collapse of mitochondrial membrane potential and reduce apoptosis activation.[80] The effects of melatonin on autophagy seem to be related to the levels of activation of catabolic processes as well as to specific cellular conditions, so that melatonin promotes the basal levels of autophagy under physiological conditions in order to maintain neuronal homeostasis and survival.[96] Accordingly, melatonin accumulates within mitochondria to prevent cardiolipin peroxidation in order to maintain cardiolipin interaction with autophagosomes via LC3II, which permits degradation of damaged mitochondria by mitophagy during aging and under neurodegenerative conditions.[97] Autophagic activation also blocks mitochondrial dysfunction and apoptotic cell death during neurotoxicity induced by human prion proteins.[81] Melatonin increases autophagy to protect neurons against mitochondrial apoptosis resulting from a subarachnoid hemorrhage.[82] Damaged mitochondria disposal by autophagy is a key protective

mechanism against neuroinflammation that occurs after traumatic brain injury.[86] Thus, melatonin activates mitophagy, via the mammalian target of rapamycin (mTOR), to attenuate inflammation induced by traumatic injury in the CNS.[86,87]

Melatonin prevents oxaliplatin-induced neuronal apoptosis by increasing the autophagy pathway (via LC3I/II) in peripheral nerves and dorsal root ganglia, thus preserving the epidermal nerve fiber density in neuropathic animal models.[98] Also, a rapid modulation of the silent information regulator 1 (SIRT1) by melatonin leads to autophagy activation to block cell death induced after a hypoxia-ischemia process in the neonatal rat brain.[99]

In response to several other specific neurodegenerative conditions, beneficial effects of melatonin are mediated by a decrease of exacerbated autophagy.[96] Via this means, melatonin protects cells from autophagic cell death triggered by the Bcl-2/Beclin1 pathway, by inhibiting the activation of c-Jun N-terminal kinase 1, the upstream pathway that leads to Bcl-2 phosphorylation and its release from the complex with Beclin1 for activating autophagy.[78] Pretreatment with melatonin also attenuates loss of dopaminergic neuronal impairments ofthe length of axons and dendrites induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), via the autophagy inhibition mediated by cyclin-dependent kinase 5 and α-synuclein aggregation. This reduces symptoms of dyskinesias in animal models of PD.[85]

Neurodegenerative drugs such as kainic acid and rotenone induce autophagic cell death associated with mitochondrial dysfunction.[100,101] Notably, melatonin has a neuroprotective role against neurotoxicity induced by kainic acid exposure via inhibition of the lysosomal-autophagy system and α-synuclein aggregation.[100] Likewise, melatonin mitigates rotenone-induced autophagic cell death via blocking dynamic-related protein 1 expression and its mitochondrial translocation.[101] Indeed, recent

studies suggest that mitophagy is functionally associated with mitochondrial dynamics, through the interaction between mitochondrial dynamics factors and LC3 adapter/receptors.[102] Likewise, mitochondrial dynamic could impact on several cell processes that include Ca^{2+} signaling, ROS generation and apoptosis,[103] with relevant consequences for the maintenance of dendritic spines and neuronal plasticity.[91] These premises suggest that mitochondrial dynamics and mitophagy affect each other to maintain the quality of mitochondria. This way, melatonin might impact on the neurodegeneration level though the regulation of mitochondrial dynamic via modulating of mitophagy.

Melatonin also blocks autophagy to ameliorate arsenite-induced neurotoxicity, thus recovering neuronal integrity and mitochondrial mass.[84] Brain injury induced by subarachnoid hemorrhage can be alleviated by melatonin suppressing excessive neuronal apoptosis and autophagy by means of targeting the pathway that involves ROS generation and the mammalian sterile 20-like kinase 1 activation.[90] Melatonin also plays a protective role against ischemia-reperfusion brain injury by limiting autophagy, due to targeting of ER stress signaling pathways[89] as well as activation of the PI3K/Akt pro-survival pathway.[83]

Herein, we have reviewed the dual effects of melatonin on autophagy by which it exerts its neuroprotective role during neurodegenerative conditions (Fig. 4). On one hand, during aging, age-related neurodegenerative diseases and neurodestructive conditions such as traumatic brain injury, brain subarachnoid hemorrhage, infection by human prions, peripheral neuropathies and hypoxia/ischemia processes, autophagy activation is linked to a neuroprotective role that maintains cell survival. Thus, under these conditions, therapeutic effects of melatonin are associated with the preservation of the protective role of autophagy. On the other hand, neurotoxitic agents such as

methamphetamines, MPTP, kainic acid, rotenone, arsenite as well as in situations of brain ischemia and reperfusion, autophagy contributes to pathology and mediates cell death. Thus, under these specific brain pathological conditions, melatonin blocks autophagy to exert its neuroprotective role against neurodegenerative damage.

CANCER

Increased levels of both ROS and antioxidants have been detected in almost all cancer cells suggesting that a particular redox status balancing ROS-mediated carcinogenesis and ROS-induced cell death is required for tumor development and progression.[104] Melatonin has a key role in the etiology and pathology of cancer as observed by many studies showing increased cancer incidence in night shift workers[105-107] and cancer patients with disturbed melatonin secretion.[108] In addition, melatonin also displays intrinsic antitumoral properties via its effects on cell cycle, apoptosis, oxidative stress, immune stimulation, and growth signals[109,110] through both receptor-mediated or direct actions with free radicals, proteins or lipids. It has been shown that melatonin represses the Warburg effect, ameliorates disturbed mitochondrial bioenergetics and is a pro-oxidant in cancer cells and even in cancer stem cells (CSCs).[111-114] However, this set of actions may not be essential for the antiproliferative effects of melatonin.[115] In this context, MT1 and MT2 receptors may also play roles in some oncostatic actions of melatonin by inhibiting the uptake of linoleic acid, a promoter of tumor growth and development.[116] Melatonin is selectively cytotoxicity towards cancer cells while protecting healthy cells; it is also crucially important to overcome drug resistance in cancer chemotherapy.[117] For example, melatonin activates proliferative Akt signaling in healthy cells but melatonin-treated cancer cells experience reduced Akt signaling.[118,119] It was hypothesized that this selectivity may be explained by a G-protein switching

between Gi and Gs.[120] MT receptors modulatePI3K/Akt signaling[121] that has an eminent role in autophagy modulation.[122] Despite this, the significance of the effects of melatonin on autophagy in cancer cells is still uncertain.

Abundant evidence indicates that alterations in the autophagic pathway can influence cell fate during cancer development and chemoresistance. Under normal conditions, cells degrade dysfunctional organelles and/or misfolded proteins by autophagy to release biomolecules for reuse. However, when cells encounter unfavorable environments, the autophagic machinery can be redirected to a mechanism of death.[123] In addition, the accumulation of misfolded proteins induces stress to the ER. It has been demonstrated that a variety of anti-cancer therapies stimulate ER stress and autophagy[124]. ER stress activates the UPR to restore ER homeostasis through adaptive mechanisms that also involve the stimulation of autophagy. However, when persistent, ER stress can switch the cytoprotective functions of UPR into cell death safeguard mechanisms.[124]

In general, it is accepted that autophagy plays a double role in tumor development: a) its activation inhibits tumor growth through the induction of bioenergetic failure and cell death. Alternatively, it can promote cell survival by preventing or eliminating lesions induced in organelles by chemotherapeutic agents. There are some randomized controlled clinical trials using combinations of autophagy inhibitors to increase the responsiveness of cancer therapeutics.[125] Furthermore, autophagy protects against cancer by mediating both innate and adaptive immune responses;[126] b) its reduction can increase genomic instability favoring carcinogenic processes. Although these paradoxical functions of autophagy remain to be fully elucidated, some observations indicate a primary role of autophagy to prevent cancer but, once a tumor develops, autophagy is then exploited by cancer cells to adapt to

stressful, nutrient- and oxygen-starved environments.[127] In addition, autophagy has been proposed to regulate stemness of cancer stem cells (CSC).[128] For example, the autophagic protein Beclin1 appears to be critical for breast CSC maintenance but also to hinder the development of breast tumors not enriched in CSC.[129] Furthermore, it has been recently noted that autophagy inhibition reduces pancreatic CSC activity and enhances the anti-tumor effect of gemcitabine.[130] While some studies suggest that the activation of autophagy induces cell death and suppresses epithelial-mesenchymal transition in cancer cells[131] and even in CSC,[132] others point to an association between hypoxia-induced autophagy, epithelial-mesenchymal transition and the metastatic potential of CSC.[133]

Melatonin modulates autophagy and exerts protective effects in several conditions either through enhancement or inhibition of the autophagy process.[134-136] In general, melatonin induces downregulation of mTOR and Akt phosphorylation in various types of cancer cells[137-139] and, as far as we know, a majority of the literature indicates that melatonin seems to promote and control autophagy in cancer cells[140] by different means depending on their ability to activate apoptosis. Thus, in some apoptosis-sensitive cells such as human myeloid HL-60 cells, melatonin induces mitochondrial-mediated apoptosis.[111] In fact, it is well established that melatonin induces apoptosis in a variety of tumor types, such as breast cancer,[141] pancreatic cancer,[142] renal cáncer,[143] neuroblastoma[144] and gastric cáncer.[145] Despite this cytotoxic action, in some cancer cells such as in breast tumor xenografts exposed to dim light exposure at night, melatonin supplementation induced autophagy leading to tumor regression.[146] In addition, in hepatocellular carcinoma cells, melatonin also enhanced mitophagy when used in combination with sorafenib, probably due to its actions in increasing mitochondrial depolarization and ROS production in cancer cells.[147] The

removal of damaged mitochondria alleviates oxidative stress and prevents carcinogenesis. In addition, the therapeutic induction of autophagy mediated by melatonin in apoptosis-defective cells usually leads to autophagic death, a type of cell death with characteristics intermediate between apoptosis and autophagy. Thus, HCT116 colorectal cancer cells treated with melatonin showed cell senescence, up-regulated Bax, cleaved-caspase 3 and LC3, as well as decreased Akt signaling, effects mediated by a MT1 receptor downregulation;[148] the contribution of autophagy in melatonin-induced cell death was also described in glioma CSC.[149]

Similar results were recently obtained in gastric cancer cells where hyperbaric oxygen treatment sensitized cells to melatonin-induced autophagic apoptosis[150] and in head and neck squamous cell carcinoma where a combined treatment with rapamycin and melatonin blocked the negative feedback loop on mTOR/Akt pathway and induced changes in mitochondrial function together with mitophagy and apoptosis;[151] This suggests an antitumor role of autophagy by promoting cell death mechanisms during synergistic anticancer therapies. Despite this, in other cases such as in liver cancer cells, melatonin inhibited mTOR/Akt pathway and induced autophagy protecting from apoptosis[138] and even, in some cases such as in HepG2 cells, it acted independently of mTOR signaling[152]. In any case, autophagy inhibition with 3-methyladenine or by Beclin1 or Atg5 knockdown enhanced the pro-apoptotic actions of melatonin suggesting that therapeutic strategies should be combined with autophagy inhibitors.[138,152]

Taking into an account the dual role of autophagy in cancer development, simultaneous autophagy activation and blockage at the autophagosome-lysosome fusion step has also been suggested as a promising approach in anticancer therapies.[153] In this context, the tacrine-melatonin hybrid C10 containing melatonin was able to increase

autophagy initiation markers while accumulating autolysosomes and inducing cellular senescence and death.[154] On the contrary, other reports have also documented anti-autophagic properties of melatonin contributing to its anticancer activity such as in hepatoma,[155] neuroblastoma[156] and in colon carcinogenesis,[157] emphasizing the role of autophagy in providing a favorable microenvironment for cancer cell survival in some stages of cancer development.

Collectively, the findings indicate that melatonin exerts a wide range of anticancer actions that might allow its interventional use in different types of cancer, while preserving cellular homeostasis in normal cells[158] (Fig. 5). However, considering the controversial role of autophagy in cancer besides the fact that melatonin can both promote and inhibit autophagy depending on the cell type, treatment conditions and tumor microenvironmental factors, it is difficult to generate a consensus about the effects of melatonin in cancer regarding autophagy. Furthermore, autophagy activation might contribute to the establishment of senescence[148,159] that might also be transient and facilitate tumor relapse.[160-162] Autophagy inhibition should be carefully considered due to the implication of different intermediates in cell survival and death decisions. Finally, more research related to the autophagic effects of melatonin on resistance to chemotherapeutics is needed.

VIRUS INFECTION

A variety of the stressful stimuli, which are triggered during different stages of viral replication, can induce autophagy. Among these, danger-associated molecular patterns (DAMPs), ER and redox stress, are noted (Fig. 6). Virus-induced autophagy is capable of preventing the early apoptotic death of cells suggesting that xenophagy, a cellular process dedicated to engulf and destroy pathogens, might limit the cytopathic

effect of viruses and the pathological consequences associated with cell death triggered by viral infection.[163]

The first mechanism to limit the extent of viral spread involves a large repertoire of pattern recognition receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMPs), such as viral nucleic acids and their synthetic analogs produced during a viral infection. These receptors include Toll-like receptors (TLRs), Nod-like receptors (NLRs), RIG-I-like receptors (RLRs), and AIM2-like receptors (ALRs).[164-166] Thus, an autophagy-dependent activation of TLR3 is shown in Coxsackie virus B3 (CVB3)-infected kidney fibroblasts.[167] Vesicular stomatitis virus (VSV)-infected plasmacytoid dendritic cells (pDCs) produce interferon-α (IFN-α) by a mechanism depending on the autophagic delivery of viral replication intermediates to TLR7 present on late endosomes. A control of VSV replication *in vivo* by autophagy is suggested by the fact that ATG5-/- VSV-infected mice exhibited elevated viral loads.[168]

A second mechanism involves selective recognition and sequestration of autophagic targets by proteins that function as autophagic adaptors. The main autophagic adaptors that are classified as sequestosome-1/p62-like receptors (SLR) in response to viral infections include p62, neighbor of BRCA1 gene 1 (NBR1), nuclear dot protein of Mr 52,000 (NDP52) and optineurin.[169] An example of this mechanism is the autophagic degradation of Sindbis virus, which involves the selective degradation of the viral capsid in a p62-dependent manner.[170]

Viral replication itself frequently elicits stress responses, such as production of ROS, RNS and reactive lipid species (RLS), which can triggered autophagic processes. A prominent redox signaling pathway responsive to ROS/RNS/RLS is nuclear factor-erythroid 2-related factor 2/Kelch-like ECH (enoyl-CoA hydratase)-associated protein 1 (Nrf2/Keap1) pathway. Nrf2 is negatively regulated by the cytoplasmic redox-sensor

protein Keap1 via its Nrf2/ECH homology 2 (Neh2) domain. Nrf2 is released by the modification of a cysteine residue of Keap1 and translocated to the nucleus where it activates the transcription of both antioxidant and autophagic genes, such as p62.[171] Thus, autophagy can be indirectly regulated by transcriptional mechanisms in response to ROS/RNS via Nrf2/Keap1 pathway. Protection against the oxidant-induced exacerbation of influenza A virus infection is led by Nrf2-mediated antioxidant pathways.[172] A role for the transcription factor Nrf2 in limiting both antiviral and cell death responses to the dengue virus (DENV) by feedback modulation of oxidative stress has been reported.[173] An increase in Nrf2-dependent antioxidants has been proposed to protect HBV-infected cells from oxidative-mediated cell damage.[174]

Other stress response which can be elicited by viral replication is the ER stress produced by an accumulation of misfolded or unfolded proteins in this compartment. ER stress activates an unfolded protein response (UPR) mediated by three ER membrane-associated proteins. HCV infection induces ER stress and activates all three sensors of UPR. A reduction of the amount of LC3-II and an inhibition in HCV replication can be obtained by silencing any the three UPR signaling proteins mediated by siRNA.[175] Mutant HCV lacking the envelope glycoproteins and subgenomic replicons expressing non-structural proteins (NS3-5B) induced autophagy in the absence of detectable UPR.[176] Nevertheless, expression of the replicase protein NS4A of other related flaviviruses, such as DENV and Modoc virus induce autophagy via UPR.[177] Abundant VZV glycoprotein biosynthesis triggers UPR via ER-stress and induces autophagy to maintain cellular homeostasis.[171]

Although autophagy is a mechanism which can limit the cytopathic effect of viruses and the pathological consequences, some viruses have developed strategies to directly or indirectly subvert autophagy in order to promote different stages of the viral

life cycle. Most positive-strand RNA viruses trigger autophagy to re-shape the endomembrane system in order to create complex membranous replication factories.[178] Double-membrane vesicles (DMVs), which are characteristic of autophagosome-like structures, were detected in cells infected by several picornaviruses, such as enterovirus 71 (EV71), coxsackievirus B3 (CVB3), poliovirus and foot-and-mouth disease virus (FMDV). Moreover, expression of the picornaviral proteins 2BC and 3A mediates the formation of autophagosome-like DMVs induced by the lipidation of LC3.[179] In some cases a decrease in virus production is observed in absence of autophagy.[180]

Autophagy is blocked at the final degradative steps during EBV replication allowing the virus to skip its degradation by lysosomes and enhance viral replication.[181] The replication and transcription activator (RTA), an essential viral protein for Kaposi's sarcoma-associated herpes virus (KSHV) lytic reactivation, enhances the autophagic process, leading to an elevation in the number of autophagic vacuoles, an increase in the level of the lipid bound form of LC3 protein, and the formation of autolysosomes. Moreover, the inhibition of autophagy affects RTA-mediated lytic gene expression and viral DNA replication.[182]

In enveloped viruses, viral genome must be packaged into a virus particle, which is surrounded by a double membrane envelope that is released from the infected cell. Since autophagosomes might serve as a membrane source, an efficient envelopment is known to depend on induction of autophagy. Thus, major HBV envelope protein (HBsAg) binds to and co-localizes with LC3-I and LC3-II during HBV infection indicating that this interaction might be important for acquiring the viral envelope.[183] On the other hand, autophagy has been shown to serve also in the non-lytic release of non-enveloped viruses via the formation of DMVs, such as those described in the case of picornaviruses (rhinovirus 14, rhinovirus 2 or poliovirus). The presence of both VP1

(poliovirus capsid protein) and LC3 in extracellular structures adjacent to poliovirus-infected cells supports a potential mechanism for the release of cytosolic picornaviral particles via the formation of these DMVs.[184]

Melatonin induces or inhibits autophagy based on cellular necessities and oxidative stress levels. The properties of melatonin can explain the role of this indoleamine as an autophagy regulator. Thus, melatonin easily crosses morphophysiological barriers, including the placenta and the blood-brain barrier and readily enters cells.[185-187] Within cells melatonin is a powerful and effective ROS/RNS scavenger, which provides protection against oxidative damage in all cellular compartments.[11,188] Furthermore, melatonin stimulates several antioxidative enzymes including GPx, CAT and SOD, thereby potentiating its antioxidant properties.[189,190] The mechanisms by which melatonin stimulates the activities of ROS detoxifying enzymes involve the Nrf2/Keap1/ARE pathway,[191] indicating that melatonin can regulate oxidative stress-induced autophagy via Nrf2.

Melatonin also modulates the ER stress response. Cyclosporine A (CsA) is a powerful immunosuppressive drug with side effects including nephrotoxicity and neurotoxicity; these toxicities induce autophagic cell death in rat pituitary GH3 cells via depletion of catalase expression and increasing the expression of some ER stress markers (BiP and IRE-1α). Melatonin suppresses CsA-mediated autophagy in GH3 cells by elevating catalase expression and reducing of BiP and IRE-1α expression.[192] Treatment of mice with carbon tetra-chloride induced fibrosis while melatonin inhibits ER stress.[135] Thus, melatonin likely regulates the autophagic processes, which are associated with changes in the cellular production of ROS/RNS or with induction of ER stress, both of which are triggered by a viral infection (Fig. 6).

Lagoviruses, such as rabbit hemorrhagic disease virus (RHDV) and rabbit vesivirus (RaV) induce autophagosome and autophagolysosome formation.[193,194] During RHDV-induced autophagy, increased expression of Beclin1, LC3-II/LC-I ratio and Atg5-Atg12-Atg16L1, as well as colocalization of LC3 and LAMP-1, were reported. Nevertheless, a parallel rise in p62/SQSTM1 expression was also detected indicating dysfunctional autophagy with impairment of the autophagic flux.[195] Melatonin decreases RHDV-induced autophagic response by reducing oxidative stress and ER stress;[196,197] these involve a reduction in CHOP and BiP expression and a rise in the GSSG/GSH ratio, as well as on inhibition of RHDV RNA replication.[195] Melatonin administration could trigger similar mechanisms in other RNA viruses, whose infection causes a similar dysfunctional autophagy, such as HCV.[198,199]

Although oxidative stress is one of the earliest events in Alzheimer disease (AD) and plays a crucial role in the onset and development of the disease, it has been reported that HSV-1 infection can act as an inducer of the most important pathological hallmarks of AD.[200] Thus, oxidative stress potentiates the accumulation of intracellular β-amyloid peptide mediated by HSV-1 infection and further inhibits its secretion to the extracellular medium. AD is also associated with the accumulation of autophagic compartments without increasing the degradation of long-lived proteins, and enhances the inhibition of the autophagic flux induced by HSV-1.[201] Melatonin, whose beneficial role in AD has been frequently reported,[202] may regulate the neurodegenerative events associated with HSV-1 infection because of its properties as an oxidative stress inhibitor.

Chronic HCV infection predisposes patients to develop liver failure after acetaminophen (APAP) overdose. Some APAP treated-transgenic mice, which express the HCV structural proteins core, E1 and E2, experience exacerbation of all parameters

of liver injury (elevated ALT levels, focal centrilobular necrosis and nuclear DNA fragmentation), while other mice are protected. HCV transgenic mice with higher liver injury had lower liver glutathione levels, elevated mitochondrial oxidative stress and enhanced release of apoptosis-inducing factor (AIF) from the mitochondria, as well as induction of a higher ER stress response and induction of autophagy. By comparison, transgenic animals showing protection against liver injury had a robust recovery of liver glutathione content, accompanied by reduction in mitochondrial oxidative stress and AIF release. Thus, HCV infection may exacerbate APAP-induced liver injury due to induction and amplification of mitochondrial oxidant stress and it could also protect against injury by activation of APAP scavenging mechanisms.[203] Because of its marked antioxidant capability, melatonin should be evaluated in a randomized controlled trial in the cases of an exacerbated APAP-induced liver injury induced by HCV infection.

Autophagy plays an important role both in the antiviral defense responses and in the promotion of the different stages of the viral life cycle. The fact that melatonin is a regulator of autophagy due to its properties as a potent antioxidant and suppressor of ER stress, suggests a potential beneficial role for this molecule in the management of some viral infections. This opens the possibility to extend similar studies to other viruses, whose biological cycle or antiviral treatments involve autophagic processes.

OBESITY

Obesity is caused by a diet rich in energy together with a sedentary life; this condition has become an epidemic health problem in developed countries. Its main risk is the development of obesity-related comorbidities that include cancer, cardiovascular diseases, diabetes, and/or metabolic syndrome, where several conditions such as hypertension, lipidemia, high blood glucose level and high cholesterol are clustered.

The metabolic syndrome, whichis the cause of heart diseases and/or type 2 diabetes, reduces significantly the quality of lifeand favors the fragility of a high percentage of patients. All of this make obesity one of the five leading causes of global deaths.[204] It is necessary to understand the molecular mechanisms that underlie the development of obesity to avoid its fatal consequences. However, obesity is a complicated issue since many causes can induce the development of this pathology from genetic, physiological to nutritional obesity origins.[205] This makes obesity a multifactorial disease and difficult to understand.

Extreme obesity, known as morbid obesity, is the most lethal phase of this disease and one of the less studied.[206] In most of the cases, a reduction inleptin levels and/or a development of leptin resistance are the basis. Leptin is an adipokine of 16-Kda[207] that acts at central nervous system level, regulating appetite, metabolism and sexual maturation.[208] This peptide, which is secreted by peripheral adipocytes,acts on its receptorswithin the hypothalamus.[209] Alterations in this signaling mechanism induce "munchies" that leads quickly to obesity. But even isolating one of the main causes of obesity, such as absence of leptin is difficut to evaluate since there are differential actions in different organs. Thus, it is necessary to analyze common effects that can be uniformly found at organic level in obesity and later dwell on the divergences at the organic level.

One of the effects most commonly associated with obesity is the high production of free radicals. Obesity is usually considered as a state of chronic oxidative stress,[210] where maintained food intake causes important damage, mainly at mitochondrial level[211] which induces exacerbated ROS production. This extreme oxidative stress is responsible for excessive oxidation of glucose and lipids. It is a proven fact in overweight,[212] obesity,[213] but, especially in morbid obesity, risk factor for related

comorbidities is exponentially increased.[214] Likewise, the increase in ROS generation has a strong inflammatory aspect, especially as adipocytes release proinflammatory cytokines.[215,216]

The oxidative damage observed in obesity is striking and a small number of trials of melatonin administration on this condition have been described, which take into account its recognized role as antioxidant.[217] Moreover, several articles have related melatonin with weight loss promotion by stimulating thermogenesis, recruitment of brown fat[218] and change in body weight.[219] Also, a relationship between leptin and melatonin has been described.[220] Leptin can also have a chronobiotic role, since this peptide is secreted with circadian rhythmicity, with a maximal production in the middle of the night.[209] Thus, leptin-signaling disruption alters circadian rhythms. Moreover, melatonin and leptin are involved in common processes, as adipose tissue deregulation and chronic inflammation;[220] melatonin increases leptin concentration after oral administration.[221] In fact, for these reasons, melatonin could be considered an adequate molecule to protect against the damaging effects of obesity.[222]

Another effect that has been recently related to obesity, regardless of organ studied, is autophagy. Autophagy as a mechanism implies the maintenance of cellular homeostasis by recycling damaged macromolecules and organelles, regulation of energy imbalance and neurohormonal dysregulation that are present in obese subjects.[223] However, obesity processes alter autophagy in different ways depending on the organ studied.[224] It is, therefore, necessary to contextualize the damage by assessing the possible factors involved in the observed alterations.

The multifactorial characteristics that this disease has, together with expressed contextualization have stimulated, in many cases, studies based on animal models. Among them, the leptin-deficient mouse is especially useful for developing morbid

obesity.[225] Commonly referred as ob/ob, these animals carry a mutation in leptin gene that results in a nonfunctional leptin, and loss of the feeling of fullness, which makes these animals hyperphagic and obese. Different researchers have studied the organic damage at the cellular level under melatonin treatment (500µg/kg).[226,227] Comparison of these articles clearly shows that the effects are organ-dependent (Fig. 7). Thus, a liver from a leptin-deficient mouse showed an increase of proteolytic processes, as autophagy of a proteasome, to counteract a large rise of unfolded proteins that the hepatocytes presented.[222,226] These damaged proteins are the result of high ER stress due to hyperphagia. However, detailed studies of the autophagic data related to aggregate markers such as p62 indicated that autophagy blockage occurred; this prevented degradation of damaged macromolecules. These events accelerate the cascade of widespread damage with increased oxidative stress, subsequently associated inflammation, hyperglycemia and an increase in insulin receptor expression, which, given the difficulty of producing correct proteins, could be considered a diabetes risk marker.

Melatonin administration ameliorated several symptoms observed in the liver of ob/ob mice; not only was oxidative stress reduced, but also inflammation and ER stress. The decline in unfolded protein production is a relevant result which leads to improvement in other mechanisms dependent on it. Reducing damaged macromolecules leads to a decline in the blockage. Simultaneously, with melatonin administration oxidative stress is reduced as is the associated inflammation. The role of melatonin in this case is unquestionable and beneficial. Other authors have previously reported that melatonin administration induces amelioration of obesity characteristics with increased antioxidant enzyme activity and a reduction of body weight.[228,229] Leptin absence induces hyperphagia that is not reduced by melatonin administration. Therefore, in this

special case, melatonin did not reduce body weight, but improved the general state of the liver. Thus, the described beneficial characteristics were obtained at time that ob/ob plus melatonin mice consumed 26% more food than control animals. Even with this extra food consumption, animals did not show differences from ob/ob controls. This finding highlights the important role that melatonin has in increasing the efficiency of metabolic processes.[226]

The role of melatonin in ameliorating and preventing deleterious effects induced by obesity and especially morbid obesity has been a goal of several studies that have used ob/ob animals. Considering the close relationship that has been described between oxidative stress and obesity and the susceptibility of the CNS to oxidative stress, the damage to the brain due to high glucose and oxygen consumption is of special interest. It has been recently documented that there is a rise in oxidative stress and ER stress with the accumulation of unfolded proteins that trigger autophagy in ob/ob mice.[227] This over-stimulation of autophagy could not be indefinitely maintained, which led to the accumulation of misfolded proteins.

The brain, however, has its own particularities, and thus, ER-stress could not trigger the unfolded protein response blocking possible systemic improvements. Subsequent unfolded proteins induce aggresomes formation and accumulation of neurodegenerative markers, including β-amyloid, α-synnuclein and tau hyperphosphorylation, causing the appearance of anxiety and stress-induced behavioral changes.[227] In that organ, melatonin administration counteracted all the damaging effects: improved antioxidant defenses, reduced ER-stress and misfolded proteins; this in turn, led to a reduction in autophagy and proteasome and neurodegenerative markers were reduced and a normal behavior was restored.[227] Analogous results were seen in damaged structures as a consequence of fructose consumption with improvements

caused by melatonin administration.[8] Although fructose was used as sweetener for diabetics based on its low glycemic index, its harmful effects at the multiorganic level soon became apparent. Its pattern of toxicity is very similar to that observed in obesity: increase in oxidative stress, accumulation of aggregates but an inability to trigger the ER stress response. This causes an impairment of the macroautophagic machinery and the development of neurodegenerative markers including tau hyperphosphorylation. Melatonin reduced these harmful effects in fructose-treated mice as it did in ob/ob mice.

In skeletal muscle, the results observed are similar to those in the CNS. Thus, the absence of leptin induced lipid anabolism and stimulated an excessive oxidative phosphorylation that led to mitochondrial damage in muscle. These important muscle alterations resulted in myofiber degeneration and oxidative type I fiber conversion. Melatonin treatment induced an improvement in regulation of energy homeostasis and mitochondrial function, restoring mitochondrial bioenergetic regulation and mimicking leptin signaling as seen under physiological functions (Data unpublished).

These results document the powerful therapeutic role that melatonin could have for multiorgan recovery of individuals who are overweight,[230,231] obese[232] or morbidly obese.[226,227,233] Melatonin is a very potent antioxidant and since oxidative stress is an early and important consequence of faulty energy metabolism, accumulation of unfolded proteins and mitochondrial dysfunction due to uncontrolled oxidative phosphorylation, it seems essential that melatonin can be used as a powerful therapeutic agent against the deleterious effects of obesity.

The consequences of eating disorders leading to obesity are exaggerated in old individuals. There are few obese elderly people the multiorgan degeneration caused by severe obesity makes the association between obesity and aging less common. Unfortunately, however, overweight and alterations in energy metabolism are common

in the elderly and they have been widely studied, especially at the level of the brain.[234] In this organ, oxidative stress is elevated merely due to aging itself. ER-stress, autophagy blockage and, eventually, mitochondrial dysfunction in extreme cases is due to unfolded proteins and mitochondria dysfunction.[235]

A recent study carried out in humans described the harmful effects that overweight induces in aged people.[59] Oxidative stress, inflammation, ER-stress, inefficient autophagy and insulin resistance were observed in the muscle of these individuals. Depletion of myogenic regulatory factors seems to suggest a reduction of satellite cells in muscle tissue. Thus melatonin may be a useful agent against these harmful effects.

CONCLUSIONS

Although melatonin´s role in the prevention and protection in biological processes as different as those described in this review are clearly documented, the molecular mechanisms to explain its function are poorly studied. Likewise, many factors trigger autophagy, which involve multiple steps and types; this makes it difficult to understand the role of autophagy in each process. Thus, to clarify the context or circumstances where autophagy comes into play is essential to identify the consequences of its alteration. Thus, we consider that any study of the relationship of these mechanisms of cell cleaning and survival must occur, in any pathology, by placing it in its corresponding environment.

On the basis of data discussed herein, it's clearly shown that one of these essential mechanisms involved in the beneficial role of melatonin is autophagy, whose alterations, whether due to excess or a defect in activity, have been clearly documented. Melatonin can regulate autophagy both directly by modulating its activity and

improving the proteolysis pathway, and indirectly by either reversing mitochondrial dysfunction due to a reduction of an excessive oxidative stress, resulting in ATP production increase, or by improving ER efficiency, resulting in a reduction in the amount of misfolded proteins. The concrete molecular mechanisms by which melatonin regulate autophagy are described in the different situations reviewed in this manuscript and shown in table 1. In summary, melatonin has a beneficial role on a wide range of disorders acting as an autophagy regulator following different mechanisms, such as those explained in this review.

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FIGURE LEGENDS

Figure 1. Overview of the molecular and cellular events during autophagy. Autophagy begins with the formation of the phagophore (initiation/nucleation step). The phagophore expansion leads to the formation of an autophagosome (elongation/closure step). During this step, the autophagosome can engulf bulk cytoplasm non-specifically, including entire organelles, or target cargos specifically. The outer membrane of the autophagosome can fuse with an endosome (forming an amphisome before fusing with the lysosome; not shown) or directly with a lysosome to form an autophagolysosome (degradation step). Finally, the sequestered material is degraded inside the autophagolyosome and recycled.

Figure 2. Diagram of the UPR arms and their connection to autophagy. UPR is mediated by three ER membrane-associated proteins [PERK, ATF6 and IRE1], which are normally inactivated by the chaperone BiP. The interaction of BiP with unfolded luminal proteins, whose accumulation cause ER stress, activates PERK, ATF6 and IRE1 and culminates the transcription of autophagy-related genes via activation of XBP1, CHOP, and ATF4.

Figure 3. Mitochondrial function and autophagy regulation by melatonin as a protector against aging-causing damage.

Figure 4. Dual effects of melatonin on autophagy to exert a neuroprotective role under neurodegenerative conditions.

Figure 5. Schematic representation of autophagy-related antitumoral and pro-tumoral effects of melatonin during cancer development supporting the use of combined anticancer strategies based on simultaneous autophagy induction and blockade. CSCs, cancer stem cells.

Figure 6. Viral manipulation of autophagy. Viral infection can trigger several mechanisms, such as interaction with cell surface receptors and autophagic adaptors and oxidative and ER stress induction. These mechanisms can induce autophagy, which can be inhibited at early or late stages of the pathway by certain virus to promote its replication. Role of melatonin as a regulator of autophagy due to its properties as potent antioxidant and suppressor of ER stress supports the potential beneficial role for this molecule in the management of some viral infections.

Figure 7. Cell effects of obesity on liver, brain and muscle and recovery by melatonin. Mainly, melatonin is able to reduce damage by ROS reduction, mitochondria protection and minimization of unfolded proteins. All these effects, collaborate to autophagy improvement

Table 1. Mechanisms whereby melatonin regulates autophagy

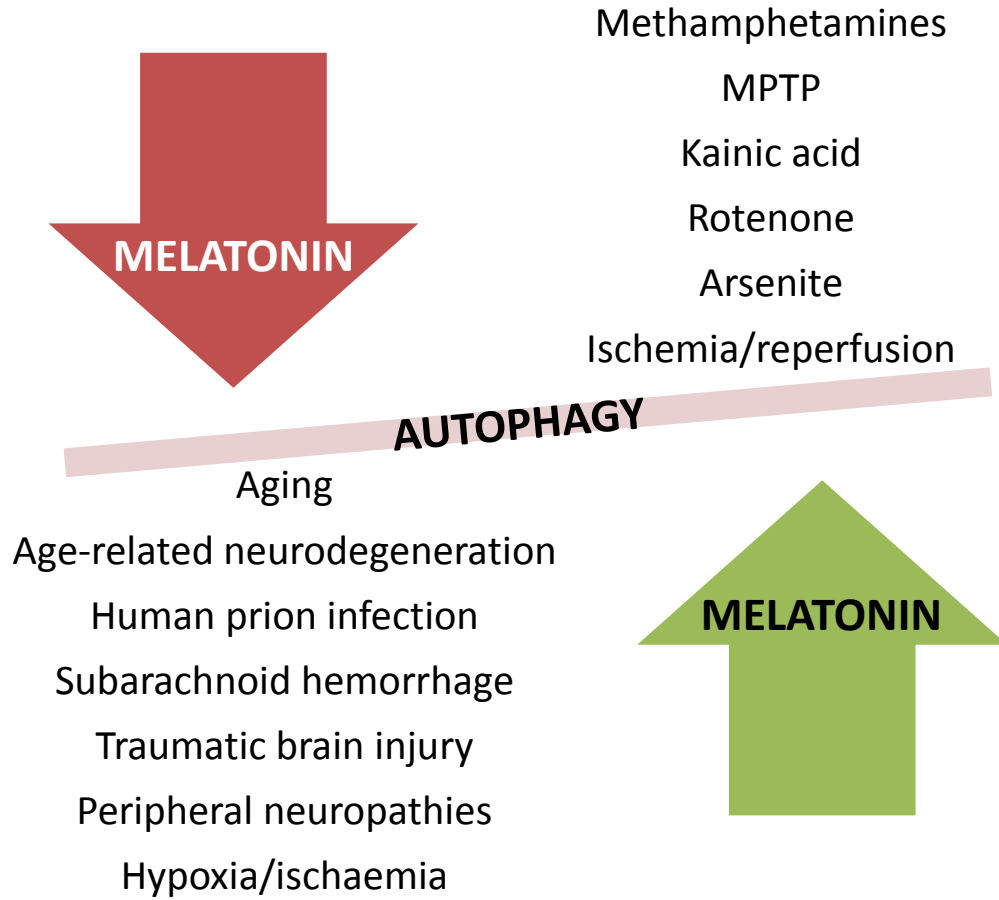| Autophagy	Mechanism	Condition/pathology	References
Induction	mTOR/Akt inhibiton	Cancer	Liu et al. 2012
			Martin et al. 2014
			Shen et al. 2018
		Neurodegeneration	Jenwitheesuk et al., 2014
			Ding et al., 2015
			Lin et al., 2016
	Circadian rhythm restoration	Cancer	Dauchy et al. 2014
	Reactive oxygen species (ROS) production	Cancer	Prieto-Dominguez et al. 2016
	Antioxidant-mediated effect	Neurodegeneration	Phillipson, 2017
	Mitochondria-mediated effect	Cancer	Prieto-Dominguez et al. 2016
			Wei et al.2018
		Neurodegeneration	Jeong et al., 2012
			Chen et al., 2014
			Ding et al., 2015
	MT1 receptor downregulation	Cancer	Hong et al.2014
	ER stress-mediated effect	Cancer	Ordonez et al.2015
		Obesity	de Luxan-Delgado et al., 2016
			Wei et al.2018
	SIRT 1 modulation	Neurodegeneration	Carloni et al., 2017
		Aging	Nopparat et al., 2017
	Mitochondrial membrane rigidity reduction	Aging	García et al., 2011
Inhibition	Modulation of the Bcl-2/Beclin 1 complex	Neurodegeneration	Nopparat et al., 2010
	CDK-5 modulation	Neurodegeneration	Su et al., 2015
	Antioxidant-mediated effect	Neurodegeneration	Chang et al., 2012
		Virus Infection	San Miguel et al., 2014
	Mitochondrial dinamic regulation	Neurodegeneration	Zhou et al., 2018
	Regulation of ROS-MST1 pathway	Neurodegeneration	Shi et al., 2018
	ER stress-mediated effect	Neurodegeneration	Feng et al., 2017
		Obesity	Rubio-Gonzalez et al., 2018
		High fructose consumption	Bermejo-Millo et al., 2018
		Virus Infection	San Miguel et al., 2014
	Activation of PI3K/Akt pro-survival pathway	Neurodegeneration	Zheng et al., 2014

Figure 1
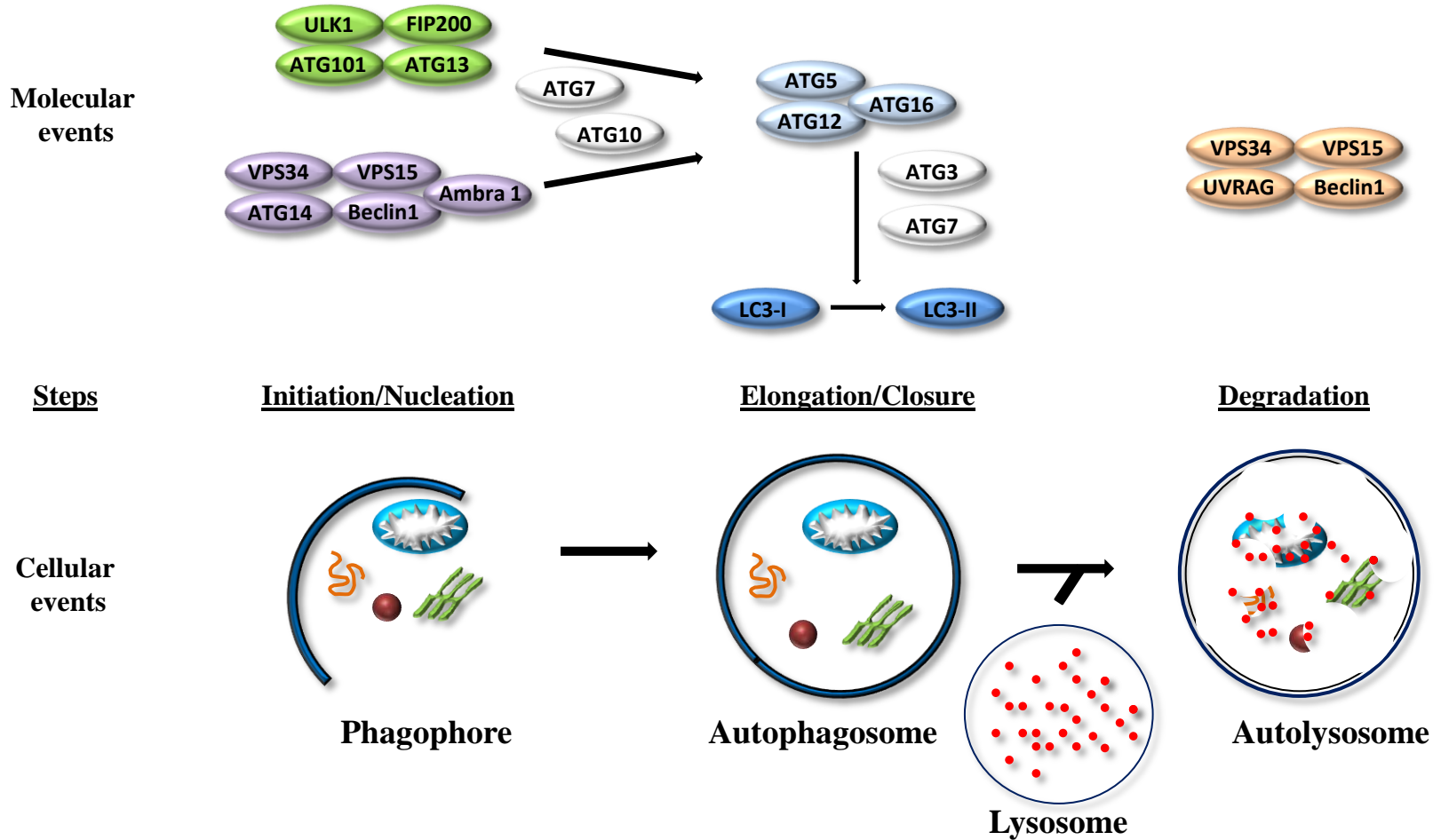
Figure 2
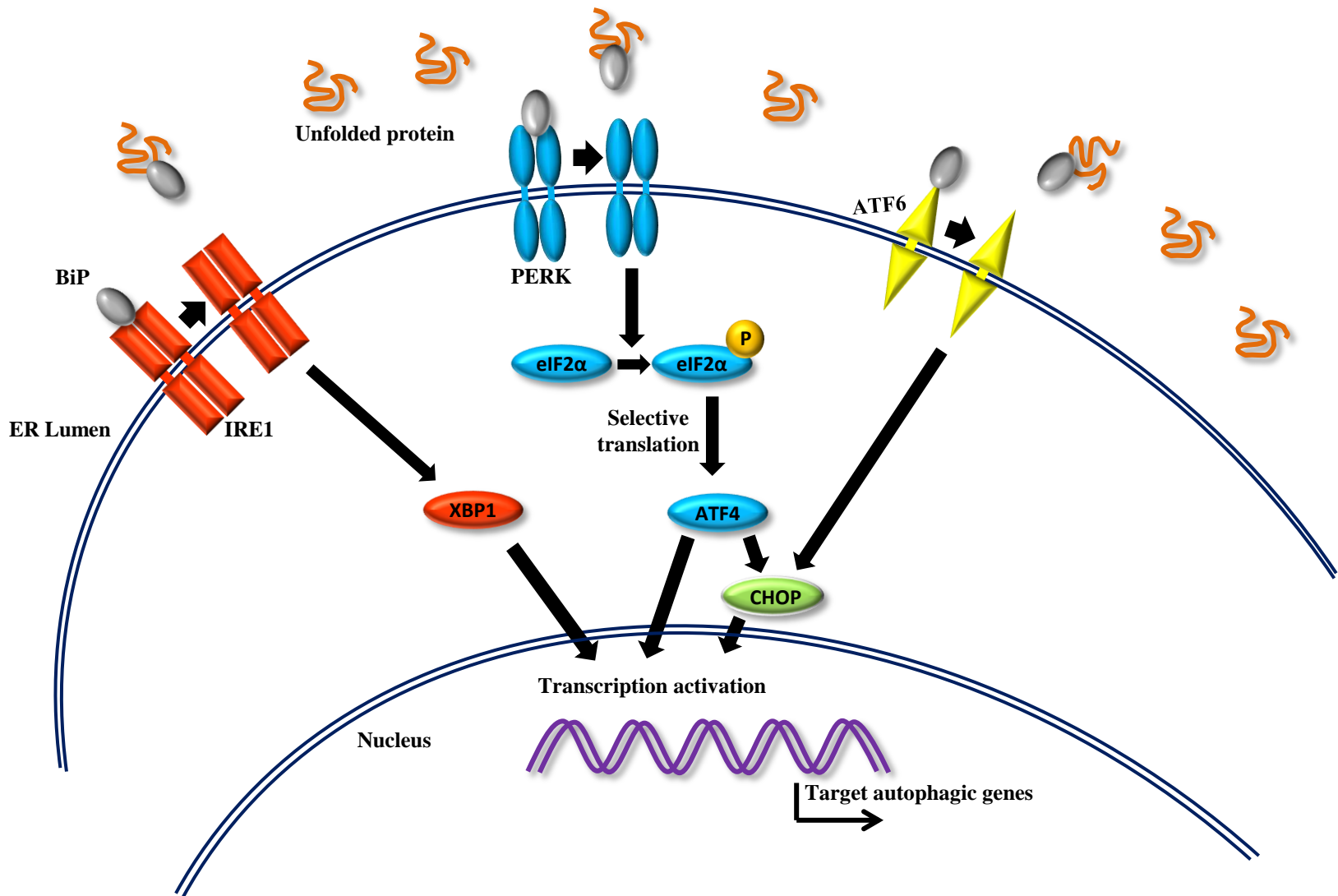
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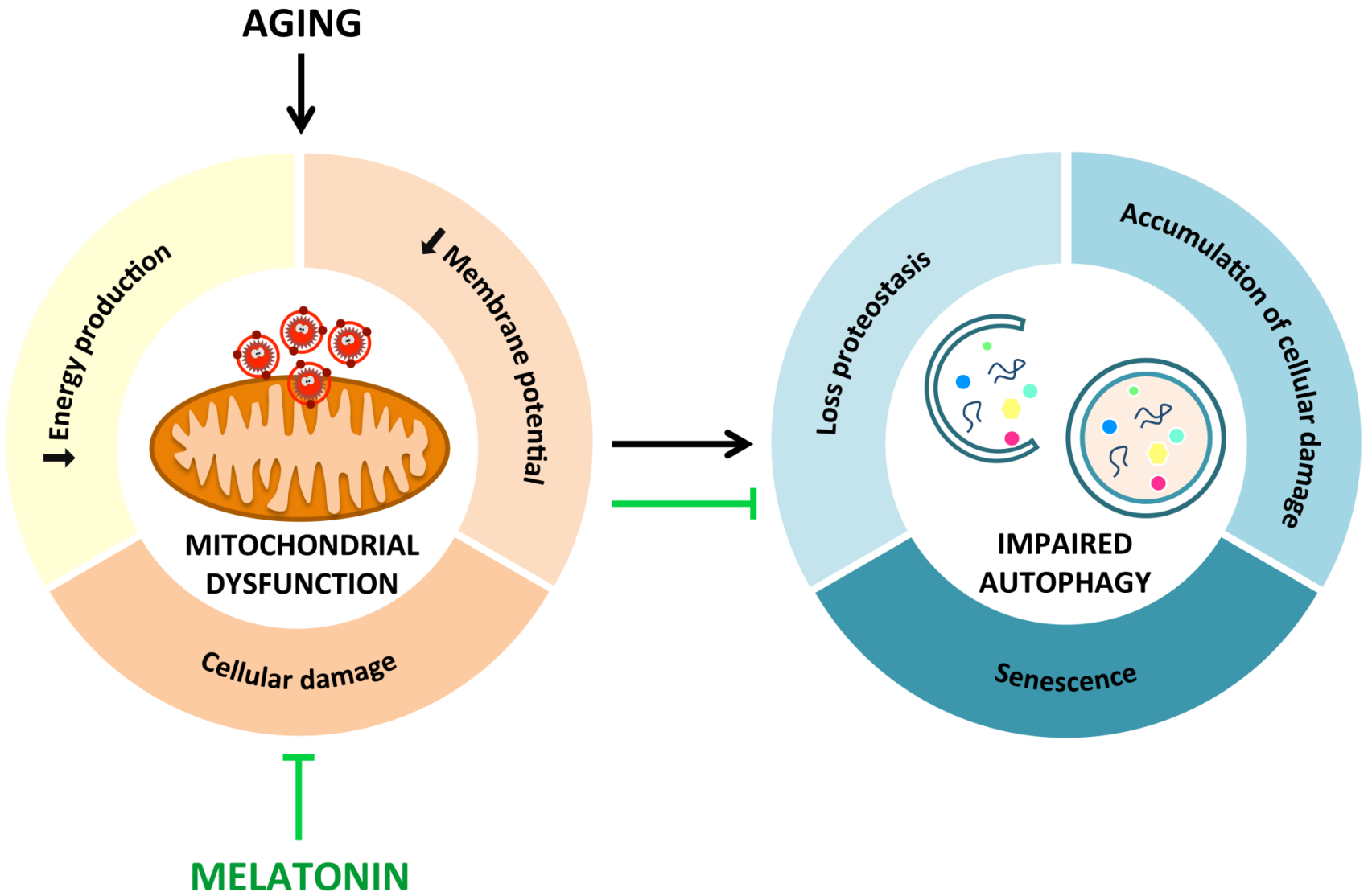
Figure 4

Figure 5

Figure 6

Figure 7