Review

Melatonin and its ubiquitous effects on cell function and survival: A review

Wilson MT. Kuwabara[^1], Patricia RL. Gomes[^1], Jéssica Andrade-Silva[1], José Maria Soares Jr[1]., Fernanda G. Amaral[2], José Cipolla-Neto[*1].

[1]Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil
[2]Department of Physiology, Federal University of São Paulo, São Paulo, Brazil
[^]Contribute equally
[*]Correspondence: cipolla@icb.usp.br, Tel +551130917466.

Running title: Melatonin and cell function and survival

Received: June 2, 2022; Accepted: June 29, 2022

ABSTRACT

Melatonin, a phylogenic conserved molecule, presents in almost all living organisms and it is believed to be originated to protect the unicellular organisms from oxidative products which were emerged from aerobic respiration. Even with the acquisition of a variety of other functions along evolution, the crucial autocrine, paracrine and endocrine actions of melatonin in the regulation of cell biology were well preserved. The molecular mechanisms involved in the cell cycle that determine survival and death need to be tightly regulated. Changes in these mechanisms can trigger pathologies that compromise the entire balance of the body. In this context, melatonin acts on cellular homeostasis by regulating the main molecular mechanisms that sustain life and control death, such as synthesis and degradation of protein, energy supply and pathways which trigger death to remove the defective cell or any microorganism from the tissues. Thus, this review aims to briefly present the action mechanisms of melatonin, in addition to discussing its fundamental role in cellular processes such as synthesis and degradation of protein, mitochondrial function and cell death control.

Key words: melatonin, cell biology, cell death, mitochondria

---

1. MELATONIN, A BRIEF REVIEW

Melatonin (N-acetyl-5-methoxytryptamine) is an indolamine with a molecular mass of 232 which presents three basic properties: 1) it is amphiphilic, being almost equally diffusible in aqueous and in lipid medium so that it can be found in all compartments of the cells, 2) it is a chronobiotic hormone and 3) it is a powerful antioxidant molecule, efficiently scavenging almost any oxygen species (ROS) and nitrogen reactive species (RNS). Its primary function seems to be linked to its antioxidant property which is well preserved throughout the phylogenetic tree and its several other cellular and systemic functions were acquired during evolution, according to the complexity of the organism and to the site of melatonin production.

Melatonin is a phylogenic conserved molecule outlasting by far the origin of the pineal gland, that is present in vertebrates, as its origin can be traced back to the initial aerobic metabolism, probably 2.5 billion years ago (1-3). Being present in every existing taxon, melatonin can be found in prokaryote and eukaryote, unicellular or pluricellular organisms

such as: archaea, bacteria, algae, protozoa, fungi, plants, invertebrates, and vertebrates (4-6). In most of these organisms, melatonin acts as a chemical mediator with autocrine and paracrine signaling properties due to its diffusion characteristics. Its actions as a hormone (endocrine signaling) are mainly observed in vertebrates. In mammals, synthesized from the amino acid tryptophan as the primary substrate, melatonin can be produced by several peripheral tissues for local use, in addition of being produced by the pineal gland and released in the circulation, acting as a hormone (7-9). The pineal gland is an epithalamic structure, originating from the roof of the third ventricle and lying over the quadrigeminal mesencephalic lamina. This particular anatomical location determines that pineal melatonin is not only directly released to the blood stream, but it is also especially released into the cerebrospinal fluid of the third ventricle, reaching the central nervous system (CNS) (10). Therefore, the CNS is one of the main targets of melatonin actions, consequently interfering with main physiological systems such as endocrine system, energy metabolism (14-16), cardiovascular system (10, 17-19), activity-wake/rest-sleep cycle (10, 20-22), reproduction (23-27).

Due to the control by a unique neural pathway in mammals, pineal melatonin synthesis is circadian and seasonally rhythmic, being exclusively allocated to dark phase of the environmental light-dark cycle, independently of the activity/rest circadian profile of the given species (diurnal, nocturnal or crepuscular) (10). It is important to notice that the night-restricted melatonin production takes place only if the night is dark, i.e., there is no light in the environment. Light at night, especially in the blue/green range, inhibits melatonin production, being able to completely block it depending on its intensity and time of exposure (28, 29). Moreover, the nocturnal duration of melatonin production precisely follows the duration of the dark phase of the light-dark cycle, that is strictly dependent on the seasons of the year. These characteristics of melatonin determine its most essential function in the regulation of vertebrate physiology: melatonin nocturnal profile internally represents the external photoperiodic daily and seasonal geophysical cycle. In accordance, pineal melatonin profile serves as the interface between the environmental light-dark cycle and the chemical signaling the day and night and the different seasons to the internal environment of the organisms to trigger all the corresponding physiological and behavioral adaptations (29).

The question that follows is how melatonin endocrine signal can transmit this information about the lightning condition of the external environment to the internal target cells, organs, and systemic physiology. It is usually expected that a chemical messenger, especially a hormone, signals through specific receptors that define its target element's function. In fact, there are two specific melatonin membrane receptors, nominated as MT1 (encoded by *MTNR1A gene*) and MT2 (encoded by *MTRN1B gene*) (30). They are G protein-coupled receptors linked to G_i / G_o or G_q / G_{11}, signaling, therefore, through the inhibition of cAMP or cGMP synthesis and/or activation of phospholipase C, depending on the central or peripheral localization of the targets. It should be mentioned that melatonin can, as well, regulate the transduction pathway of nuclear receptors of the family of the retinoic acid (ROR/retinoid Z receptor) in certain systems.

Moreover, due to its phylogenetic origin and its amphiphilicity, melatonin can act independently of receptors, in an intracellularly direct action (31). In this case, in addition to direct scavenging free radicals, melatonin can interact with other important substances including calcium/calmodulin dependent kinase II and several intracellular proteins (32). As mentioned above, the well-known antioxidant effect of melatonin is, in part, dependent on its direct interaction with ROS and RNS, besides upregulating expressions of antioxidant enzymes in a receptor-mediated action (33). Some of these actions of melatonin are the common features of hormones generally and others are unique to melatonin acquired during evolution such as being an internal signal to inform the changes of the external day and night and the seasons of the year to the organisms, especially to mammals (10). As similar to other hormones,

melatonin presents what is known as immediate effects due to its direct interaction with its molecular acceptors (receptor and non-receptor mediated effects described above). These effects are observed on any target system during the night, when melatonin is present in the circulation and in the cerebrospinal fluid. In addition, some effects of melatonin only occur after the cessation of melatonin signaling, in the following day. These are the so-called prospective effects which can be classified to two types. 1) Proximal or consecutive effects, that appear immediately after the end of melatonin production (during the morning of the next day) and last for some hours and it is dependent on the hypersensitivity or supersensitivity of the intracellular adenylyl cyclase/cAMP/PKA/CREB transduction pathway that appears after the withdraw of the long-lasting nocturnal inhibition due to the interaction of melatonin and Gi-dependent receptors. The withdraw of this long-lasting nocturnal inhibition allows a potentiation of the action of any Gs-linked receptor agonist that mobilize adenyl cyclase-cAMP production, as seen in several central and peripheral systems (suprachiasmatic hypothalamic nucleus, pituitary pars tuberalis, pancreatic beta cell, Leydig cells, ovary cells, etc.) (10). 2) Distant or prolonged effects, that appear at any time during the following day, provided melatonin is not present. They are dependent on melatonin regulation of the expression of the so-called clock genes. Once triggered, the expression of these genes lasts for approximately 24 hours. These clock genes, in turn, control the expression of the clock-controlled genes that are the functional genes controlling cellular functions.

In addition, due to its regularity of production, clear contrast between night and day and clear relationship to the external photoperiod, melatonin is able to synchronize the circadian oscillators, either central or peripheral. Acting on central circadian clock, hypothalamic suprachiasmatic nucleus, melatonin regulates its phase and period contributing importantly to the circadian temporal structure of the physiological and behavioral processes (10). In this way, night-restricted melatonin release determines effects that are seen all over the 24 hours either during the night (immediate effects) or during the following day (prospective consecutive or prolonged effects) allowing melatonin to internally signals the external night and day and, as a circadian chronobiotic agent participating decisively in the circadian organization of physiology and behavior.

As far as the seasonal signaling is concerned the involved mechanisms are different. First, as previously mentioned, the internal nocturnal profile of melatonin strictly follows the external duration of the dark night (34, 35). This duration of nocturnal melatonin production is detected by a special central structure called pituitary *pars tuberalis* (36). This structure control local thyroid stimulating hormone (TSH) production depending on the duration and on the direction of changing (increasing or decreasing) of melatonin nocturnal profile. However, when this signal is translated into physiological and/or behavioral modifications that are adaptive to the different seasons of the year, it must be transmitted from the pituitary *pars tuberalis* to the hypothalamus, that is the behavior-physiology integrative center of the CNS. This is accomplished by hypothalamic-third ventricle special radial glial cells, the tanycytes, whose cells bodies line the ventral portion of the third ventricle, interposed to the ependymal cells, and present cellular processes projecting to several hypothalamic nucleus, median eminence and *pars tuberalis* (37). TSH receptors are present in the membrane of the tanycytes reaching the *pars tuberalis*. These receptors are activated by paracrine signaling of the *pars tuberalis* cells that release TSH in the extracellular space. Tanycytes' TSH receptor-mediated transduction pathway activates the expression of type 2 iodothyronine deiodinase (DIO2) and reduces the expression of type 3 iodothyronine deiodinase (DIO3). DIO2 is the enzyme responsible for converting T4 to T3, the active form of the hormone. DIO3 inactivates thyroid hormone by converting T4 into reverse T3, an inactive form. In summary, the duration of the nocturnal profile of melatonin mediated by pituitary *pars tuberalis* and hypothalamic tanycytes

regulates the availability of active T3 in the hypothalamus, that is known to control the seasonal fluctuation of physiology and behavior (10, 38, 39).

Considering the mechanisms of action of melatonin, it is known that it is involved in cellular functioning through several putative mechanisms (10, 38, 39). Whether via pineal melatonin or via melatonin produced locally by cells, for example in mitochondria, there will be direct (autocrine action) or indirect (paracrine or endocrine actions) regulation on various cellular functions. The role played by melatonin in the regulation of protein homeostasis, mitochondrial function and cell death is going to be discussed below.

2. EFFECTS OF MELATONIN ON THE SYNTHESIS AND DEGRADATION OF PROTEINS

Melatonin regulates protein turnover by controlling protein synthesis and degradation. As it is well-known, protein synthetic machinery is dependent on three major organelles: endoplasmic reticulum (ER), mitochondria and Golgi complex (GC). The balance between their function and crosstalk are essential to produce biologically active proteins. All proteins transiting the secretory pathway in eukaryotic cells first enter the ER, undergo conformational changes, form complexes and are exported into vesicles to the Golgi apparatus for subsequent secretion (40). During protein synthesis, there is a process of quality control that prevents unfolded protein formation by binding them to chaperones, preventing their exportation to the GC; and also directing these malformed proteins to the cytoplasmic 26S proteasome for degradation and elimination through the process called ER-associated degradation (ERAD) (41).

The ER lumen has a unique highly oxidizing environment that favors the protein folding from the very beginning, when the nascent polypeptide chain enters the organelle (40). High protein concentration and/or the presence of unfolded proteins increase the probability of forming protein aggregates that can trigger and sustain the ER stress. Specific ER proteins such as the 78kDa and 94kDa glucose-regulated proteins (GRP78/Bip; GRP94) are responsible for binding to these unfolded proteins, therefore, preventing the emergence of protein clusters and the resultant stress response, reestablishing the organelle homeostasis (42). Furthermore, the ER is the main store of calcium in the cell necessary for the synthesis of new biologically active proteins, in addition to ATP. The energy provided by ER-associated mitochondria is essential not only for protein folding but also for maintaining chaperone function, intraluminal calcium ion levels, the redox state of ER, and protein degradation by ERAD (43). Thus, any impairment in mitochondrial function can disrupt ER homeostasis and trigger the stress response. Therefore, the accumulation of unfolded proteins initiates the activation of a stress signaling cascade known as the Unfolded Protein Response (UPR). ER stress activates stress-sensing transducers: Inositol-Requiring kinase 1 (IRE1), protein kinase-like ER kinase (PERK) and Activating transcription factor 6 (ATF6) (44).

When UPR is activated, IRE1 autophosphorylates its cytoplasmic domain to activate its ribonuclease function. Activated IRE1 cleaves the X-box binding protein mRNA (XBP1) to generate the spliced form of XBP1s which triggers the transcription of several genes involved in the UPR (44). In addition, by processing XBP1 mRNA, IRE1 directly and indirectly promotes the rapid breakdown of several mRNAs associated with the rough ER, decreasing the entry of new protein to the lumen of this organelle. When chronically activated, IRE1 binds to Tumor Necrosis Factor receptor-associated factor 2 (TRAF2) activating the pro-apoptotic kinase JUN N-terminal kinase (JNK) through the apoptosis signal-regulating kinase 1 (ASK1) (45). Activated JNK triggers cell death by apoptosis or autophagy. The IRE1 activity is regulated by the interaction with BAX inhibitor 1 and proteins of the Bcl-2 family (44).

PERK dissociates from chaperone GRP78 when unfolded protein increases in the lumen of the ER, dimerizes, phosphorylates and consequently inactivates the eukaryotic translation factor 2α (eIF2α) (46, 47). Phospho-eIF2α abolishes the translation and overall protein synthesis to prevent the ER overload. However, some protein synthesis is still sustained during PERK activation (47). Activating Transcription Factor 4 (ATF4) is activated by phospho-eIF2α (48) and increases the transcription of some genes involved in the stress response, such as chaperones (GRP78 and GRP94), and of genes involved in the suppression of oxidative stress, metabolism and transport of amino acids. ATF4 also activates the CCAAT/enhancer-binding protein homologous protein (CHOP), which triggers ER associated apoptosis when the UPR is chronically activated (49). ATF6 remains in the ER during normal conditions in association with GRP78 (50). When the unfolded protein concentration increases in the ER lumen, ATF6 dissociates from the chaperone and translocates to the Golgi apparatus where it is cleaved by site-1 (S1P) and site-2 (S2P) proteases, releasing the 50 kDa cytosolic domain (p50ATF6) as an active transcription factor. p50ATF6 activates the transcription of several genes involved in ER quality control, including chaperones such as GRP78, XBP1, ERAD components of the apoptosis-inducing CHOP and Endoplasmic reticulum resident oxidoreductase 1β (Ero1β) that has a key role in maintaining the oxidative environment of the ER (50, 51).

The endoplasmic reticulum is associated with mitochondria in regions called Mitochondria-Associated ER Membrane (MAM). The MAM has fundamental interest in cellular homeostasis including non-vesicular transport of phospholipids, calcium ion transport from the ER to the mitochondria to stimulate oxidative metabolism and the control of calcium concentration in the ER lumen (41, 44, 52, 53). Any change in the mitochondrial function and, therefore, in the MAM domain formation is associated with an increased rate of cell death and the development of various diseases, such as Parkinson, Alzheimer, inflammatory and metabolic related pathologies (41, 44, 52, 54-57). Prolonged UPR activation is correlated to several pathologies, such as type 2 diabetes, cancer, viral infections, neurodegenerative, cardiovascular and inflammation related diseases (44, 58, 59). Melatonin is known to regulate ER stress by modulating the action of the three ER stress sensors (60) PERK is known to play an important role in the ER stress-induced apoptosis due to heart and brain ischemia when there is a cell depletion of ATP because of the lack of oxygen supply, which consequently leads to an accumulation of unfolded protein in the ER lumen and UPR activation. Melatonin protects from heart injury caused by ischemia-reperfusion by reducing PERK-eIF2α-ATF4 pathway and consequently decreasing the stimulus to induce cell death (61). In the brain, melatonin protects from injury also by reducing PERK phosphorylation (activation), phospho-eIF2α and consequently decreasing the content of cleaved-caspase 3 and cytochrome C. Melatonin-treated animals presented lower brain infarction volumes, lesion sites and increased number of surviving neurons (62). On the other hand, melatonin seems to increase ER stress and activate CHOP, leading to increased apoptosis and autophagy in cancer cells (63, 64). IRE1-α is also modulated by melatonin. Central insulin resistance (IR) and/or cerebral ischemic stroke led to ER stress activation that causes cell death. Melatonin decreases the content of p-IRE1 and spliced XBP1, protecting neurons from death (65). ATF6 is blocked by melatonin, which in turn inhibits cyclooxygenase-2 (COX-2) and leads human hepatoma cells to ER stress inducing apoptosis. Melatonin was also able to protect against neuronal apoptosis by suppressing the ATF6/CHOP pathway (66). In addition to interfering with protein synthesis, melatonin also appears to regulate protein degradation. Due to the similarities between proteasome inhibitors and melatonin in nuclear factor 2 related to erythroid factor 2 (Nrf2), nuclear factor kappa B (NFκB) influencing the balance of protein 2 in B-cell lymphoma (Bcl-2) and Bax, melatonin inhibits ubiquitin ligase complex Cul3-Rbx1 (E3 ligase) and the proteasome itself. By modulating this organelle, melatonin can modify cellular redox state, mitochondrial biogenesis and cell death (67).

3. MITOCHONDRIA AND MELATONIN

Every step during the cellular life cycle requires energy. Mitochondria is responsible for generating energy in eukaryotic aerobic cells by a very specific mechanism. ATP, a chemical energy carrier, is produced in mitochondria inner membrane by a sequence of electron transporter proteins, known as electron transport chain (ETC) (68). Basically, proteins transfer electrons one to another and at the end of the chain, oxygen is the final electron acceptor and generates H_2O as a result. This electron transport generates a proton gradient between the inner membrane and matrix, which drives hydrogen ions back to the matrix through ATP synthase. As it happens, ATP synthase forms ATP from ADP and inorganic phosphate (P_i) (68). In the ETC, electrons can escape the regular chain flux, especially when the cell is metabolic activated, and form free radicals as they react with molecular oxygen. These free radicals, such as superoxide anion, react with other molecules and form different types of more dangerous radicals, for instance, hydroxyl radical and peroxynitrite. These ROS trigger mitochondrial oxidative stress and may damage the organelle, releasing some apoptotic molecules, such as cytochrome C, which in turn activates caspase 3 and consequently the death process (69, 70).

On other hand, ROS are not always detrimental for the organelle and the cell since these species also participate in several important signaling pathways, such as ion transport, inflammation and protein degradation). Damaged and dysfunctional mitochondria is eliminated by mitophagy to maintain cell homeostasis. Mitophagy is the specific mitochondrial degradation through autophagic process and it plays an important role in the cell life cycle since it protects the cell from the activation of mitochondrial death pathways, once this organelle is damaged, and also enhances mitochondrial turn over (73).

Mitochondrial fusion/fission is also important for the energy and metabolic status of the cell and consequently for its homeostasis (74). Mitochondria are dynamic organelles with the ability to fuse (fusion) and divide (fission) depending on the physiological condition of the cell. Mitochondrial fusion is associated with enhanced ATP production and mitochondrial function, while excessive fission is considered prejudicial to the cell since it is associated to increased ROS production and impaired mitochondrial function (75, 76). For instance, hyperglycemia enhanced mitochondria fission and ROS production in cardiomyocytes (77) and myocardial contractile dysfunction is directly associated with an increase in mitochondria fission in diabetic patients (78). Dynamin-related protein 1 (Drp1) is a GTPase that mediates mitochondria fission by promoting membrane constriction and stabilizing structural intermediates of membrane division (79). On the other hand, impairment in mitochondria fusion is associated with several human pathologies, such as Alzheimer's and Parkinson's diseases. Mitofusin-1 and 2 (MFN1 and MFN2) (80) and optic atrophy protein 1 (OPA1) (81) are the proteins that allow mitochondria to completely fuse by interconnecting inner and outer membranes from two different organelles (65). Therefore, mitochondria are the powerhouse of the cell and any disturbance in its machinery can lead to cell death.

Melatonin is known to accumulate in mitochondria protecting this organelle from the oxidative stress. It was recently described that mitochondria synthetize and release melatonin and express its receptor MT1 in the outer membrane. When activated, this receptor inhibits stress-mediated cytochrome C release and block caspase 3 activation, protecting the cell from death (82). Melatonin prevents oxidative stress by inhibiting NO synthase (NOS) isoforms (83) and downregulating iNOS expression in several cell types, including microglia, astrocytes and immune cells, thereby reducing the levels of •NO and the generation of highly detrimental RNS (84). Furthermore, melatonin acts as a direct ROS/RNS scavenger by neutralizing the hydroxyl radical (•OH) (85) and indirectly by generating metabolites that can enhance its antioxidative power, constituting a radical-scavenging cascade able to eliminate 10 times more free radicals

by one melatonin molecule (86). Also, melatonin enhances the disposal of reduced glutathione (GSH) and the activity of other antioxidant enzymes.

Overall, in physiological conditions, melatonin increases the electron flux by upregulating the activity of the ETC complexes. However, under oxidative stress, melatonin is able to reestablish the electron flux and prevent mitochondria damage (68). Melatonin also regulates mitochondrial fission by decreasing the expression of Drp-1 (87) and influences mitochondrial fusion by increasing the expression of OPA1 (88). Both melatonin actions are associated to improvements of cardiac dysfunction in diabetic patients (88). Finally, melatonin induces mitophagy by upregulating Nrf2 and protects cell from mitochondria-induced death (89). Enhanced mitophagy by melatonin is associated to better prognosis in atherosclerosis (89) and brain injury (90).

4. CELL DEATH AND MELATONIN

Cell death is as important as other cell functions such as cell division, protein/lipid synthesis, migration and others, whilst it regulates cell number, tissue size and plays an important role in the cell quality control, eliminating components that can threaten homeostasis (91).

Apoptosis is a type of programmed non-inflammatory cell death that participates in several physiological processes such as embryogenesis, metamorphosis and in the defense line of the organism by eliminating infected/damaged cells (92). Apoptotic cells are characterized by the formation of cell surface protuberances (blebs) and condensation of the nucleus and cytoplasm, leading to decreased cell volume and disintegration of cellular junctions (93). In addition, the membrane externalization of phosphatidylserine is a hallmark of the early apoptosis allowing macrophages, which express phosphatidylserine receptors, to recognize apoptotic cells and eliminate them, preventing the release of inflammatory factors (94). Apoptosis can be triggered by two different pathways: 1) the intrinsic pathway, which is activated in response to cellular stress, and 2) the extrinsic pathway, activated by external stimulus from other cells and toxins. The intrinsic pathway is characterized by mitochondrial permeabilization that involves members of the B-cell lymphoma protein 2 (Bcl2) family and release of cytochrome C into the cytoplasm, which ends up in pro-caspase 9 cleavage and finally caspases 3 and 7 activation. Extrinsic pathway is initiated by tumor necrosis factor receptors (TNFRs) or the first apoptosis signal receptor (Fas), also known as cluster of differentiation 95 or apoptosis antigen-1 (CD95 or Apo-1). Following receptor activation, pro-caspases 8 and 10 are cleaved, allowing the cleavage and activation of caspases 3 or 7 (92, 93, 95).

Autophagy is another type of programmed cell death. In the autophagic process, cells catabolize damaged cellular components to reuse essential components such as carbohydrates, proteins and lipids, for cell repair and energy generation. Autophagy is triggered when the cell is under stress or starvation conditions, preventing cell damage and increasing survival chances when there is a lack of energy and nutrients (96). Autophagy is initiated by the recruitment of autophagy related proteins (ATGs) and Beclin-1 to a specific subcellular location for the autophagosome formation, a double membrane vesicle which traps the damaged organelle or any other cytosolic material, that is completed after elongation and expansion of the ATG initiated membrane. This process is controlled by Unc-51-like kinase (ULK), phosphatidylinositol 3-kinase (PI3K), ATG12-ATG5-ATG16L1 complexes and microtubule-associated protein light chain 3 (LC3) family members (97, 98). When autophagic process fails to reestablish the energy status of the cell or it is blocked by inhibitors or any other factors, cell death is triggered (97).

Pyroptosis is also a type of programmed cell death resulting in cell lysis, tissue inflammation and leukocyte recruitment. The key enzyme in this type of death is caspase-1 that

activates the cytokines IL-1β and IL-18 responsible for recruiting macrophages and polymorphonuclear cells to the infection site (99). Caspase-1 is also related to the formation of pores in the cell membrane, allowing an influx of extracellular ions and promoting cell swelling and lysis. Pyroptosis is controlled by a multiprotein complex known as inflammasome which is composed by a sensor called the nucleotide-binding oligomerization domain (NOD)-like receptor, an adapter molecule containing C-terminal caspase-recruitment domain (CARD), Apoptosis-associated speck containing CARD (ASC) and caspase-1 (100). When inflammasomes bind to pathogen-associated molecule patterns (PAMPs) they promote pro-caspase 1 activation, cytokines production and consequently death (99). A key executioner protein of pyroptosis is gasdermin D (GSDMD). Activated caspases cleave GSDMD which mediates pore formation and initiates death by pyroptosis (101).

Cell death is not always controlled and programmed. Necrosis is a type of cell death that results in the autolysis of the cell by external factors such as toxins, trauma or infection (102). Necrosis promotes rupture of cell membrane and consequent release of cell content, triggering an inflammatory process in the surrounding tissue, causing damage and leukocyte recruitment. Oxidative stress, severe energy depletion and high concentrations of cytoplasmic calcium can induce cell necrosis (103-105).

Melatonin is known as a cell death modulator. In physiological conditions, melatonin acts by protecting cells from death during metabolic disturbances. On the other hand, in diseases such as cancer, melatonin triggers apoptosis and, acts as an oncostatic molecule in a variety of tumors (106). Melatonin can regulate both intrinsic and extrinsic pathways of apoptosis. As already mentioned, melatonin protects mitochondria from oxidative stress by scavenging ROS and enhancing the ETC, which prevents the opening of membrane pores and the release of cytochrome C and caspase 3 activation (68). Also, melatonin can positively regulate the apoptosis extrinsic pathway in cancer development by modulating death receptors such as Fas (107). For instance, in human leukemia Molt-3 cells and ovarian cancer melatonin increases caspase 3 activity and, consequently, triggers cell death (108). Moreover, melatonin can inhibit human breast carcinoma proliferation by activating the p53-p21WAF1 pathway, inducing a cell cycle arrest (109). Contrary to that, melatonin inhibits caspase 3 activation in thymocytes when treated with dexamethasone (110) and, likewise, inhibits caspase 9 and 3 activity in human leukocytes in a calcium-induced apoptosis model (111). The mechanisms of melatonin action in different cells, with different metabolic profiles and with such a wide spectrum of action are still not completely understood. Melatonin membrane receptors, such as MT1 and MT2, were once thought to be involved in these different types of action. However, as melatonin is an amphiphilic molecule and can freely cross the barriers of cytoplasm and nucleus membranes, direct non-receptor mediated actions should also be taken into consideration. Moreover, melatonin is also produced from inside the cells, by mitochondria, and plays distinct roles apart of activating cell membrane receptors. Therefore, the mechanisms by which melatonin responds in different ways, depending on the cell status.

Autophagy is also highly regulated by melatonin. This molecule can act directly in the autophagic process or indirectly, by resolving mitochondria oxidative stress and reestablishing the energy supply. Melatonin can act as a double sword in the autophagic process, by inhibiting or inducing it, depending on the cell state. After traumatic brain injury, melatonin induces autophagy by enhancing Beclin-1 and LC3-II protein content. In this case, melatonin protects neurons from apoptosis due to the reestablishment of cellular energy status and to the protective effects on mitochondria (112). In hepatoma H22 tumor-bearing mice, melatonin also increased Beclin-1 and LC3-II protein content by inhibiting the phosphorylation of the mammalian target of the rapamycin (mTOR), a classic autophagy inhibitor. By doing so, melatonin prevented H22 cells from apoptosis and contributed to tumor growth (113). Moreover, liver virus infection, such as rabbit hemorrhagic disease virus (RHDV) and the hepatitis C virus, is known

to trigger ER stress and associated autophagy. Melatonin inhibited apoptosis and modulated the autophagic process by inhibiting Beclin-1 expression and LC3 conversion in the RDHV model (114).

Pyroptosis is also a target for melatonin modulation. In obese subjects, pyroptosis augments inflammation in the adipose tissue in response to high fat diet-induced obesity. Melatonin was able to inhibit inflammasome-induced pyroptosis by blunting NLRP3 activation due to LPS stimulus and by decreasing NF-κB signaling. It was observed that melatonin regulated IRF7, impeding the cytoplasmic pore formation by GSDMD (115). Moreover, melatonin also blocks pyroptotic death in endothelial cells by blunting the NLRP3 activation via miR-223 inhibition. These findings suggest that melatonin could be a new strategy in the treatment of atherosclerosis endothelial inflammation (116).

Considering the associations among melatonin, NLRP3 and miRNA-223, information on liquid-liquid phase separation (LLPS) and the formation of membraneless organelles (MLOs) may offer a new perspective on the mechanisms used by melatonin to suppress NLRP3 via miRNA-223.In order to down-regulate NLRP3, miRNA-223 needs to be transported by exosomes to the target site (117). However, before miRNA-223 can be transported by exosomes to specific sites, miRNA-223 must be sorted into exosomes by Y-box binding protein 1 (YBX1). For this, YBX1 must first pass through LLPS (in vitro and in cells) to form MLOs and capture miRNA-223 (118). Therefore, the MEG3/miR-223/NLRP3 axis (119) is really dependent on the proper assembly of YBX1 associated MLOs that can capture and package miRNA-223 into exosomes. More importantly, this whole process is fine-tuned and facilitated by the addition of melatonin which can regulate LLPS and the formation of MLOs.

Finally, necrosis is also modulated by melatonin. Melatonin can either inhibit or induce necrosis depending on cell types and status. For instance, melatonin enhances necrosis in pancreatic cancer cells via Bcl-2/Bax modulation (119). On the other hand, after ischemia/reperfusion, melatonin inhibits necrosis preventing tissue injury and dysfunction in pancreas and liver (120, 121). Therefore, melatonin has a dual effect on cell death processes. It depends whether the cell is harmed or if it has a defect in the dying process, like in cancers for example. Further mechanistic studies are needed to understand these differences in actions. By unveiling these concepts, melatonin would be more eligible and trusted as a molecule for treating pathologies that are triggered by dysregulation of cell death.

5. CONCLUSION AND OUTLOOK

Melatonin, a ubiquitous molecule in living organisms, appears to be an essential element in the regulation of cell biology. The complexity of it signaling, depending mainly on its origin of production and mechanisms of action, transforms melatonin into a functional cell regulator that can adjust cellular metabolism. However, all the resulting effects of melatonin that regulate cellular homeostasis trigger molecular mechanisms that sustain life and control death.

ACKNOWLEDGMENTS

This study was supported by the São Paulo Research Foundation (FAPESP), grants 2019/24327-5 and 2021/09923-0.

AUTHORSHIP

WMTK, PRLG and JAS drafted the manuscript, FGA edited and revised the manuscript, JCN outlined and wrote the manuscript, and supervised all the work. All authors revised the final version.

CONFLICT OF INTEREST

The authors declare that this review was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Tan DX, Manchester LC, Liu X, Rosales-Corral SA, Acuna-Castroviejo D, Reiter RJ (2013) Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes. *J. Pineal Res.* **54**:127-138, doi:10.1111/jpi.12026.
2. Manchester LC, Coto-Montes A, Boga JA, Andersen LP, Zhou Z, Galano A, Vriend J, Tan DX, Reiter RJ (2015) Melatonin: An ancient molecule that makes oxygen metabolically tolerable. *J. Pineal Res.* **59**: 403-419. doi:10.1111/jpi.12267.
3. Reiter RJ, Rosales-Corral S, Tan DX, Jou MJ, Galano A, Xu B (2017) Melatonin as a mitochondria-targeted antioxidant: one of evolution's best ideas. *Cell Mol. Life Sci.* **74**: 3863-3881. doi:10.1007/s00018-017-2609-7.
4. Tan DX, Hardeland R, Manchester LC, Paredes SD, Korkmaz A, Sainz RM, Mayo JC, Fuentes-Broto L, Reiter RJ (2010) The changing biological roles of melatonin during evolution: from an antioxidant to signals of darkness, sexual selection and fitness. *Biol. Rev. Camb. Philos. Soc.* **85**: 607-623, doi:10.1111/j.1469-185X.2009.00118.x.
5. Tan DX, ReiterRJ (2018) Mitochondria: The birth place, battle ground and the site of melatonin metabolism in cells. *Melatonin Res.* **2**: 44-66, doi:10.32794/mr11250011.
6. Lee K, Choi GH, Back K (2022) Functional characterization of serotonin n-acetyltransferase in archaeon thermoplasma volcanium. *Antioxidants (Basel)* **11**: (3): 596. doi:10.3390/antiox11030596.
7. Erlich SS, Apuzzo ML (1985) The pineal gland: anatomy, physiology, and clinical significance. *J. Neurosurg.* **63**: 321-341, doi:10.3171/jns.1985.63.3.0321.
8. Vernadakis AJ, Bemis WE, Bittman EL (1998) Localization and partial characterization of melatonin receptors in amphioxus, hagfish, lamprey, and skate. *Gen. Comp. Endocrinol.* **110**: 67-78, doi:10.1006/gcen.1997.7042.
9. Vollrath L (1981) The pineal organ. Springer-Verlag: Heildberg, Germany Volume VI/7, p. 659.
10. Cipolla-Neto J, Amaral FGD (2018) Melatonin as a hormone: New physiological and clinical insights. *Endocr. Rev.* **39**: 990-1028, doi:10.1210/er.2018-00084.
11. Albuquerque YML, Silva WED, Souza FAL, Teixeira VW, Teixeira Á (2020) Melatonin on hypothyroidism and gonadal development in rats: a review. *JBRA Assist Reprod.* **24**: 498-506. doi:10.5935/1518-0557.20200053.
12. Shi L, Li N, Bo L, Xu Z (2013) Melatonin and hypothalamic-pituitary-gonadal axis. *Curr. Med. Chem.* **20**: 2017-2031, doi:10.2174/09298673113209990114.
13. Khalil SS, Aziz JA, Ismail KA, El-Malkey NF (2021) Comparative protective effects of. *Can. J. Physiol. Pharmacol.* **99**: 708-719, doi:10.1139/cjpp-2020-0499.
14. Cipolla-Neto J, Amaral FG, Afeche SC, Tan DX, Reiter RJ (2014) Melatonin, energy metabolism, and obesity: a review. *J. Pineal Res.* ***56***: 371-381, doi:10.1111/jpi.12137.
15. Gomes PRL, Vilas-Boas EA, Leite EA, Munhoz AC, Lucena CF, Amaral FGD, Carpinelli AR, Cipolla-Neto J (2021) Melatonin regulates maternal pancreatic remodeling and B-cell function during pregnancy and lactation. *J. Pineal Res.* **71** (1): e12717. doi:10.1111/jpi.12717.
16. Ferreira DS, Amaral FG, Mesquita CC, Barbosa APL, Lellis-Santos C, Turati AO, Santos LR, Sollon CS, Gomes PR, Faria JA, *et al*. (2021) Maternal melatonin programs the daily

pattern of energy metabolism in adult offspring. *Plos One* **7** (6): 38795. doi:10.1371/journal.pone.0038795.
17. Baltatu OC, Senar S, Campos LA, Cipolla-Neto J (2019) Cardioprotective melatonin: translating from proof-of-concept studies to therapeutic use. *Int. J. Mol. Sci.* **20** (18): 4342. doi:10.3390/ijms20184342.
18. Baltatu OC, Amaral FG, Campos LA, Cipolla-Neto J (2017) Melatonin, mitochondria and hypertension. *Cell Mol. Life Sci.* **74**: 3955-3964, doi:10.1007/s00018-017-2613-y.
19. Campos LA, Bueno C, Barcelos IP, Halpern B, Brito LC, Amaral FG, Baltatu OC, Cipolla-Neto J (2020) Melatonin therapy improves cardiac autonomic modulation in pinealectomized patients. *Front. Endocrinol. (Lausanne)* **11**: 239. doi:10.3389/fendo.2020.00239.
20. Auld F, Maschauer EL, Morrison I, Skene DJ, Riha RL (2017) Evidence for the efficacy of melatonin in the treatment of primary adult sleep disorders. *Sleep Med. Rev.* **34:** 10-22. doi:10.1016/j.smrv.2016.06.005.
21. Dollins AB, Zhdanova IV, Wurtman RJ, Lynch HJ, Deng MH (1994) Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. *Proc. Natl. Acad. Sci. USA* **91**: 1824-1828, doi:10.1073/pnas.91.5.1824.
22. Krieg SM, Slawik H, Meyer B, Wiegand M, Stoffel M (2012) Sleep disturbance after pinealectomy in patients with pineocytoma WHO°I. *Acta Neurochir. (Wien)* **154**: 1399-1405. doi:10.1007/s00701-012-1409-y.
23. Tamura H, Nakamura Y, Korkmaz A, Manchester LC, Tan DX, Sugino N, Reiter RJ (2009) Melatonin and the ovary: Physiological and pathophysiological implications. *Fertil. Steril.* **92**: 328-343. doi:10.1016/j.fertnstert.2008.05.016.
24. Tamura H, Takasaki A, Taketani T, Tanabe M, Lee L, Tamura I, Maekawa R, Aasada H, YamagataY, Sugino N (2014) Melatonin and female reproduction. *J. Obstet. Gynaecol. Res.* **40**: 1-11. doi:10.1111/jog.12177.
25. Gomes PRL, Motta-Teixeira LC, Gallo CC, Carmo Buonfiglio DD, Camargo LS, Quintela T, Reiter RJ, Amaral FGD, Cipolla-Neto J (2021) Maternal pineal melatonin in gestation and lactation physiology, and in fetal development and programming. *Gen. Comp. Endocrinol.* **300**: 113633. doi:10.1016/j.ygcen.2020.113633.
26. Tamura H, Jozaki M, Tanabe M, Shirafuta Y, Mihara Y, Shinagawa M, Tamura I, Maekawa R, Sato S, Taketani T, *et al*. (2020) Importance of melatonin in assisted reproductive technology and ovarian aging. *Int. J. Mol. Sci.* **21** (3): 1135. doi:10.3390/ijms21031135.
27. Valias GR, Gomes PRL, Amaral FG, Alnuaimi S, Monteiro D, O'Sullivan S, Zangaro R, Cipolla-NetoJ, Acuna J, Baltatu OC, *et al*. (2022) Urinary angiotensinogen-melatonin ratio in gestational diabetes and preeclampsia. *Front. Mol. Biosci.* **9**: 800638. doi:10.3389/fmolb.2022.800638.
28. Amaral FG, Castrucci AM, Cipolla-Neto J, Poletini MO, Mendez N, Richter HG, Sellix MT (2014) Environmental control of biological rhythms: Effects on development, fertility and metabolism. *J. Neuroendocrinol.* **26**: 603-612. doi:10.1111/jne.12144.
29. Klein DC, Weller JL (1970) Indole metabolism in pineal gland - a circadian rhythm in n-acetyltransferase. *Science* **169** (3950): 1093-5. doi:DOI 10.1126/science.
30. Jockers R, Delagrange P, Dubocovich ML, Markus RP, Renault N, Tosini G, Cecon E, Zlotos DP (2016) Update on melatonin receptors: IUPHAR Review 20. *Brit. J. Pharmacol.* **173**: 2702-2725. doi:10.1111/bph.13536.
31. Reiter RJ, Tan DX, Manchester LC, Terron MP, Flores LJ, Koppisepi S (2007) Medical implications of melatonin: receptor-mediated and receptor-independent actions. *Adv. Med. Sci-Poland.* **52**: 11-28.

32. Benitez-King G, Rios A, Martinez A, Anton-Tay F (1996) In vitro inhibition of Ca2+/calmodulin-dependent kinase II activity by melatonin. *Biochim. Biophys. Acta* **1290**: 191-196.
33. Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ (2007) One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and nitrogen species? *J. Pineal Res.* **42**: 28-42. doi:10.1111/j.1600-079X.2006.00407.x.
34. Bartness TJ, Powers JB, Hastings MH, Bittman EL, Goldman BD (1993) The timed infusion paradigm for melatonin delivery - what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses. *J. Pineal Res.* **15**: 161-190.
35. Macchi MM, Bruce JN (2004)) Human pineal physiology and functional significance of melatonin. *Front. Neuroendocrinol.* **25**: 177-195. doi:10.1016/j.yfrne.2004.08.001.
36. Barrett P, Bolborea M (2012) Molecular pathways involved in seasonal body weight and reproductive responses governed by melatonin. *J. Pineal Res.* **52**: 376-388. doi:10.1111/j.1600-079X.2011.00963.x.
37. Lewis JE, Ebling FJP (2017) Tanycytes as regulators of seasonal cycles in neuroendocrine function. *Front. Neurol.* **8**: 79. doi:10.3389/fneur.2017.00079.
38. Dardente H (2012) Melatonin-dependent timing of seasonal reproduction by the pars tuberalis: pivotal roles for long daylengths and thyroid hormones. *J. Neuroendocrinol.* **24**: 249-266. doi:10.1111/j.1365-2826.2011.02250.x.
39. Dardente H, Hazlerigg DG, Ebling FJ (2014) Thyroid hormone and seasonal rhythmicity. *Front. Endocrinol. (Lausanne)* **5**: 19. doi:10.3389/fendo.2014.00019.
40. Voeltz GK, Rolls MM, Rapoport TA (2002) Structural organization of the endoplasmic reticulum. *EMBO Rep*. **3** (10): 944-50. doi: 10.1093/embo-reports/kvf202.
41. Wu X, RapoportTA (2018) Mechanistic insights into ER-associated protein degradation. *Curr. Opin. Cell Biol.* **53**: 22-28. doi:10.1016/j.ceb.2018.04.004.
42. Schröder M, Kaufman RJ (2005) The mammalian unfolded protein response. *Annu. Rev. Biochem.* **74**: 739-89. doi: 10.1146/annurev.biochem.73.011303.074134.
43. Ma Y, Hendershot LM (2001) The unfolding tale of the unfolded protein response. *Cell* **107** (7): 827-30. doi:10.1016/S0092-8674(01)00623-7.
44. Hetz C, Zhang K, Kaufman RJ (2020) Mechanisms, regulation and functions of the unfolded protein response. *Nat. Rev. Mol. Cell Biol.* **21**: 421-438. doi:10.1038/s41580-020-0250-z.
45. Tabas I, Ron D (2011) Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. *Nat. Cell Biol.* **13** (3): 184-90**.** doi:10.1038/ncb0311-184.
46. Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, Ron D (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol. Cell* **6** (5): 1099-108. doi:10.1016/S1097-2765(00)00108-8.
47. Harding HP, Zhang Y, Bertolotti A, Zeng H, Ron D (2000) Perk is essential for translational regulation and cell survival during the unfolded protein response. *Mol. Cell* **5** (5): 897-904. doi:10.1016/S1097-2765(00)80330-5.
48. Harding HP, Zhang Y, Ron D (1999) Protein translation and folding are coupled by an endoplasmic- reticulum-resident kinase. *Nature* **397** (6716): 271-4. doi:10.1038/16729.
49. Hu H, Tian M, Ding C, Yu S (2018) The C/EBP homologous protein (CHOP) Transcription factor functions in endoplasmic reticulum stress-induced apoptosis and microbial infection. *Front. Immunol.* **9:** 3083. doi:10.3389/fimmu.2018.03083.
50. Sommer T, Jarosch E (2002) BiP binding keeps ATF6 at bay. *Dev. Cell* **3** (1): 1-2**.** doi:10.1016/S1534-5807(02)00210-1.

51. Shimizu Y, Hendershot LM (2009) Oxidative folding: Cellular strategies for dealing with the resultant equimolar production of reactive oxygen species. *Antioxid. Redox Signal.* **11** (9): 2317-31. doi:10.1089/ars.2009.2501.
52. Missiroli S, Patergnani S, Caroccia N, Pedriali G, Perrone M, Previati M, Wieckowski MR, Giorgi C (2018) Mitochondria-associated membranes (MAMs) and inflammation. *Cell Death Dis.* **9** (3): 329. doi:10.1038/s41419-017-0027-2.
53. Rizzuto R, Duchen MR, Pozzan T (2004) Flirting in little space: The ER/mitochondria ca2+ liaison. *Sci. STKE* **2004** (215): re1. doi:10.1126/stke.2152004re1.
54. Area-Gomez E, Schon EA (2016) Mitochondria-associated ER membranes and Alzheimer disease. *Cur. Opi. Gene De.* **38**: 90-96, doi:10.1016/j.gde.2016.04.006.
55. Herrera-Cruz MS, Simmen T (2017) Over six decades of discovery and characterization of the architecture at mitochondria-associated membranes (MAMs). *Adv. Exp. Med. Biol.* **997**: 13-31. doi: 10.1007/978-981-10-4567-7_2.
56. Rodríguez-Arribas M, Yakhine-Diop SMS, Pedro JMB-S, Gómez-Suaga P, Gómez-Sánchez R, Martínez-Chacón G, Fuentes JM, González-Polo A, Niso-Santano M (2017) Mitochondria-associated membranes (MAMs): Overview and its role in parkinson's disease. *Mol. Neurobiol.* **54**: 6287-6303. doi:10.1007/s12035-016-0140-8.
57. Theurey P, Rieusset J (2017) Mitochondria-associated membranes response to nutrient availability and role in metabolic diseases. *Trends Endocrinol. Metab.* **28** (1): 32-45. doi:10.1016/j.tem.2016.09.002.
58. Park SW, Ozcan U (2013) Potential for therapeutic manipulation of the UPR in disease. *Semin. Immunopathol.* **35** (3): 351-73. doi:10.1007/s00281-013-0370-z.
59. Sozen E, Karademir B, Ozer NK (2015) Basic mechanisms in endoplasmic reticulum stress and relation to cardiovascular diseases. *Free Radic. Biol. Med.* **78**: 30-41. doi:10.1016/j.freeradbiomed.2014.09.031.
60. Fernández A, Ordõñez R, Reiter RJ, González-Gallego J, Mauriz JL (2015) Melatonin and endoplasmic reticulum stress: Relation to autophagy and apoptosis. *J. Pineal Res.* **59** (3): 292-307. doi:10.1111/jpi.12264.
61. Yu L, Li B, Zhang M, Jin Z, Duan W, Zhao G, Yang Y, Liu Z, Chen W, Wang S, *et al.* (2016) Melatonin reduces PERK-eIF2α-ATF4-mediated endoplasmic reticulum stress during myocardial ischemia–reperfusion injury: role of RISK and SAFE pathways interaction. *Apoptosis* **21** (7): 809-24 doi:10.1007/s10495-016-1246-1.
62. Lin YW, Chen TY, Hung CY, Tai SH, Huang SY, Chang CC, Hung HY, Lee EJ (2018) Melatonin protects brain against ischemia/reperfusion injury by attenuating endoplasmic reticulum stress. *Int. J. Mol, Med.* **42:** 182-192. doi:10.3892/ijmm.2018.3607.
63. Moreira AJ, Ordoñez R, Cerski CT, Picada JN, García-Palomo A, Marroni NP, Mauriz JL, González-Gallego J (2015) Melatonin activates endoplasmic reticulum stress and apoptosis in rats with diethylnitrosamine-induced hepatocarcinogenesis. *PLoS One* **10**: e0144517. doi:10.1371/journal.pone.0144517.
64. Zha L, Fan L, Sun G, Wang H, Ma T. Zhong F, Wei W (2012) Melatonin sensitizes human hepatoma cells to endoplasmic reticulum stress-induced apoptosis. *J. Pineal. Res.* ***52***: 322-331, doi:10.1111/j.1600-079X.2011.00946.x.
65. Song J, Kim OY (2017) Melatonin modulates neuronal cell death induced by endoplasmic reticulum stress under insulin resistance condition. *Nutrients* **9** (6): 5 93**.** doi:10.3390/nu9060593.
66. Xu W, Lu X, Zheng J, Li T, Gao L, Lenahan C, Shao A, Zhang J, Yu J (2018) Melatonin protects against neuronal apoptosis via suppression of the ATF6/CHOP pathway in a rat model of intracerebral hemorrhage. *Front. Neurosci*.**12**: 638-638.

67. Vriend J, Reiter RJ (2015) The Keap1-Nrf2-antioxidant response element pathway: A review of its regulation by melatonin and the proteasome. *Mol. Cell Endocrinol.* **401**: 213-20. doi:10.1016/j.mce.2014.12.013.
68. Hardeland R (2017). Melatonin and the electron transport chain. *Cell Mol. Life Sci.* **74** (21): 3883-3896**.** doi:10.1007/s00018-017-2615-9.
69. Murphy MP (2008) How mitochondria produce reactive oxygen species. *Biochem. J.* **417** (1): 1-13doi:10.1042/bj20081386.
70. Mitchell P (1966) Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol. Rev. Camb. Philos. Soc.* **41**: 445-502. doi:10.1111/j.1469-185x.1966.tb01501.x.
71. Shadel GS, Horvath TL (2015) Mitochondrial ROS signaling in organismal homeostasis. *Cell* **163** (3): 560-569**.** doi:10.1016/j.cell.2015.10.001.
72. Zhang J, Wang X, Vikash V, Ye Q, Wu D, Liu Y, Dong W (2016) ROS and ROS-mediated cellular signaling. *Oxid. Med. Cell Longev.* **2016**: 4350965**.** doi:10.1155/2016/4350965.
73. Lemasters JJ (2005) Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. *Rejuvenation Res.* **8** (1): 3-5.doi:10.1089/rej.2005.8.3.
74. Archer SL (2013) Mitochondrial dynamics — mitochondrial fission and fusion in human diseases. *N. Engl. J. Med.* **369** (23): 2236-51. doi:10.1056/nejmra1215233.
75. Chiong M, Cartes-Saavedra B, Norambuena-Soto I, Mondaca-Ruff D, Morales PE, GarcÃ-a-MiguelM, Mellado R (2014) Mitochondrial metabolism and the control of vascular smooth muscle cell proliferation. *Front. Cell Dev. Biol.* **2**: 72. doi:10.3389/fcell.2014.00072.
76. Ong S-B, Hall AR, Hausenloy DJ (2012) Mitochondrial dynamics in cardiovascular health and disease. *Antioxid. Redox Signal.* **19** (4): 400-14. doi:10.1089/ars.2012.4777.
77. Yu T, Sheu SS, Robotham JL, Yoon Y (2008) Mitochondrial fission mediates high glucose-induced cell death through elevated production of reactive oxygen species. *Cardiovasc. Res.* **79** (2): 341-51. doi:10.1093/cvr/cvn104.
78. Montaigne D, Marechal X, Coisne A, Debry N, Modine T, Fayad G, Potelle C, El Arid JM, Mouton S, Sebti Y, *et al*. (2014) Myocardial contractile dysfunction is associated with impaired mitochondrial function and dynamics in type 2 diabetic but not in obese patients. *Circulation* **30** (7):554-64. doi:10.1161/CIRCULATIONAHA.113.008476.
79. Ugarte-Uribe B, Müller HM, Otsuki M, Nickel W, García-Sáez AJ (2014) Dynamin-related protein 1 (Drp1) promotes structural intermediates of membrane division. *J. Biol. Chem.* **289** (44): 30645-30656. doi:10.1074/jbc.M114.575779.
80. Bach D, Pich S, Soriano FX, Vega N, BaumgartnerB, Oriola J, Daugaard JR, Lloberas J, Camps M, Zierath J.R, *et al*. (2003) Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism: A novel regulatory mechanism altered in obesity. *J. Biol. Chem.* **278** (19): 17190-7. doi:10.1074/jbc.M212754200.
81. Chen L, Liu T, Tran A, Lu X, Tomilov AA, Davies V, Cortopassi G, Chiamvimonvat N, Bers DM, Votruba M, *et al*. (2012) OPA1 mutation and late-onset cardiomyopathy: mitochondrial dysfunction and mtDNA instability. *J. Am. Heart Assoc.* **1** (5): e003012. doi:10.1161/JAHA.112.003012.
82. Suofu Y, Li W, Jean-Alphonse FG, Jia J, Khattar NK, Li J, Baranov SV, Leronni D, Mihalik AC, He Y, *et al*. (2017) Dual role of mitochondria in producing melatonin and driving GPCR signaling to block cytochrome c release. *Proc. Natl. Acad. Sci. USA.* **114** (38): E7997-E8006. doi:10.1073/pnas.1705768114.
83. Tjong YW, Li MF, Hung MW, Fung ML (2008) Melatonin ameliorates hippocampal nitric oxide production and large conductance calcium-activated potassium channel activity in

chronic intermittent hypoxia. *J. Pineal Res*. **44** (3): 234-43. doi:10.1111/j.1600-079X.2007.00515.x.
84. Escames G, López LC, Ortiz F, Ros E, Acuña-Castroviejo D (2006) Age-dependent lipopolysaccharide-induced iNOS expression and multiorgan failure in rats: Effects of melatonin treatment. *Exp. Gerontol.* **41** (11): 1165-73. doi:10.1016/j.exger.2006.09.002.
85. Poeggeler B, Saarela S, Reiter RJ, Tan DX, Chen LD, Manchester LC, Barlow-Walden LR (1994) Melatonin—A highly potent endogenous radical scavenger and electron donor: new aspects of the oxidation chemistry of this indole accessed in vitro. *Ann. N. Y. Acad. Sci.* **738**: 419-20. doi:10.1111/j.1749-6632.1994.tb21831.x.
86. Rosen J, Than NN, Koch D, Poeggeler B, Laatsch H, Hardeland R (2006), Interactions of melatonin and its metabolites with the ABTS cation radical: Extension of the radical scavenger cascade and formation of a novel class of oxidation products, C2-substituted 3-indolinones. *J. Pineal Res.* **41** (4): 374-81. doi:10.1111/j.1600-079X.2006.00379.x.
87. Ding M, Feng N, Tang D, Feng J, Li Z, Jia M, Liu Z, Gu X, Wang Y, Fu F, *et al*. (2018) Melatonin prevents Drp1-mediated mitochondrial fission in diabetic hearts through SIRT1-PGC1α pathway. *J. Pineal Res.* **65** (2): e12491 doi:10.1111/jpi.12491.
88. Ma S, Dong Z (2019) Melatonin attenuates cardiac reperfusion stress by improving opa1-related mitochondrial fusion in a Yap-Hippo pathway-dependent manner. *J. Cardiovasc. Pharmacol.* **73** (1): 27-39. doi:10.1097/FJC.0000000000000626.
89. Ma S, Chen J, Feng J, Zhang R, Fan M, Han D, Li X, Li C, Ren J, Wang Y, *et al*. (2018) Melatonin ameliorates the progression of atherosclerosis via mitophagy activation and NLRP3 inflammasome inhibition. *Oxid. Med. Cell Longev.* **2018**: 9286458. doi:10.1155/2018/9286458.
90. Sun B, Yang S, Li S, Hang C (2018). Melatonin upregulates nuclear factor erythroid-2 related factor 2 (Nrf2) and mediates mitophagy to protect against early brain injury after subarachnoid hemorrhage. *Med. Sci. Monit.* **24**: 6422-6430. doi:10.12659/msm.909221.
91. Hengartner MO (2000) The biochemistry of apoptosis. *Nature* **407** (6805): 770-6. doi:10.1038/35037710.
92. Sainz RM, Mayo JC, Rodriguez C, Tan DX, Lopez-Burillo S, Reiter RJ (2003) Melatonin and cell death: Differential actions on apoptosis in normal and cancer cells. *Cell Mol. Life Sci.* **60** (7): 1407-26. doi:10.1007/s00018-003-2319-1.
93. Ishizuya-Oka A, Hasebe T, Shi YB (2010) Apoptosis in amphibian organs during metamorphosis. *Apoptosis* **15**: 350-364, doi:10.1007/s10495-009-0422-y.
94. Vermes I, Haanen C, Steffens-Nakken H, Reutellingsperger C (1995) A novel assay for apoptosis flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *J. Immunol. Methods* **184** (1): 39-51. doi:10.1016/0022-1759(95)00072-I.
95. Nagata S (2018) Apoptosis and clearance of apoptotic cells. *Annu. Rev. Immunol.* **36**: 489-517. doi:10.1146/annurev-immunol-042617-053010.
96. YuL, Chen Y, Tooze SA (2017) Autophagy pathway: Cellular and molecular mechanisms. *Autophagy* **14** (2): 207-215. doi:10.1080/15548627.2017.1378838.
97. Dikic I, Elazar Z (2018) Mechanism and medical implications of mammalian autophagy. *Nat. Rev. Mol. Cell Biol.* **19** (6): 349-364**.** doi:10.1038/s41580-018-0003-4.
98. Tatagiba Kuwabara WM, Curi R, Alba-Loureiro TC (2017) Autophagy is impaired in neutrophils from streptozotocin-induced diabetic rats. *Front. Immunol.* **8**: 24. doi:10.3389/fimmu.2017.00024.
99. Bergsbaken T, Fink SL, Cookson BT (2009) Pyroptosis: Host cell death and inflammation. *Nat. Rev. Microbiol.* **7** (2): 99-109. doi:10.1038/nrmicro2070.
100. Malik A, Kanneganti TD (2017) Inflammasome activation and assembly at a glance. *J. Cell Sci.* **130** (23): 3955-3963. doi:10.1242/jcs.207365.

101. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, Zhuang Y, Cai T, Wang F, Shao F (2015) Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* **526** (7575): 660-5. doi:10.1038/nature15514.
102. Berghe TV, Linkermann A, Jouan-Lanhouet S, Walczak H, Vandenabeele P (2014) Regulated necrosis: The expanding network of non-apoptotic cell death pathways. *Nat. Rev. Mol. Cell Biol.* **15** (2): 135-47. doi:10.1038/nrm3737.
103. Choi K, Kim J, Kim G, Choi C (2009) Oxidative stress-induced necrotic cell death via mitochondira-dependent burst of reactive oxygen species. *Curr. Neurovasc. Res.* **6** (4): 213-22. doi:10.2174/156720209789630375.
104. Kushnareva Y, Newmeyer DD (2010) Bioenergetics and cell death. *Ann. N. Y. Acad. Sci.* **1201**: 50-57. doi: 10.1111/j.1749-6632.2010.05633.x.
105. Shaheen M, Cheema Y, ShahbazAU, Bhattacharya SK, Weber KT (2011) Intracellular calcium overloading and oxidative stress in cardiomyocyte necrosis via a mitochondriocentric signal-transducer-effector pathway. *Exp. Clin. Cardiol.* **16** (4): 109-15.
106. Reiter RJ, Rosales-Corral SA, Tan DX, Acuna-Castroviejo D, Qin L, Yang SF, Xu K (2017) Melatonin, a full service anti-cancer agent: Inhibition of initiation, progression and metastasis. *Int. J. Mol. Sci.* **18** (4): 843. doi:10.3390/ijms18040843.
107. García-Santos G, Martin V, Rodríguez-Blanco J, Herrera F, Casado-Zapico S, Sánchez-Sánchez AM, Antolín I, Rodríguez C (2012) Fas/Fas ligand regulation mediates cell death in human Ewing's sarcoma cells treated with melatonin. *Br. J. cancer* **106:** 1288-1296. doi:10.1038/bjc.2012.66.
108. Perdomo J, Cabrera J, Estévez F, Loro J, Reiter RJ, Quintana J (2013) Melatonin induces apoptosis through a caspase-dependent but reactive oxygen species-independent mechanism in human leukemia Molt-3 cells. *J. Pineal Res.* **55** (2): 195-206. doi:10.1111/jpi.12062.
109. Cos S, Mediavilla MD, Fernández R, González-Lamuño D, Sánchez-Barceló EJ (2002) Does melatonin induce apoptosis in MCF-7 human breast cancer cells in vitro? *J. Pineal Res.* **32** (2): 90-6doi:10.1034/j.1600-079x.2002.1821.x.
110. HoijmanE, Rocha Viegas L, Keller Sarmiento MI, Rosenstein RE, Pecci A (2004) Involvement of Bax protein in the prevention of glucocorticoid-induced thymocytes apoptosis by melatonin. *Endocrinology* **145** (1): 418-25. doi:10.1210/en.2003-0764.
111. Espino J, Bejarano I, Redondo PC, Rosado JA, Barriga C, Reiter RJ, Pariente JA, Rodríguez AB (2010). Melatonin reduces apoptosis induced by calcium signaling in human leukocytes: Evidence for the involvement of mitochondria and bax activation. *J. Membr. Biol.* **233** (1-3): 105-18. doi:10.1007/s00232-010-9230-0.
112. Ding K, Xu J, Wang H, Zhang L, Wu Y, Li T (2015) Melatonin protects the brain from apoptosis by enhancement of autophagy after traumatic brain injury in mice. *Neurochem. Int.* **91**: 46-54. doi:10.1016/j.neuint.2015.10.008.
113. Liu C, Jia Z, Zhang X, Hou J, Wang L, Hao S, Ruan X, Yu Z, Zheng Y (2012) Involvement of melatonin in autophagy-mediated mouse hepatoma H22 cell survival. *Int. Immunopharmacol.* **12** (2): 394-401. doi:10.1016/j.intimp.2011.12.012.
114. San-Miguel B, Crespo I, Vallejo D, Álvarez M, Prieto J, González-GallegoJ, Tuñõn MJ (2014) Melatonin modulates the autophagic response in acute liver failure induced by the rabbit hemorrhagic disease virus. *J. Pineal Res.* **56** (3): 313-21. doi:10.1111/jpi.12124.
115. Liu Z, Gan L, Xu Y, Luo D, Ren Q, Wu S, Sun, C (2017) Melatonin alleviates inflammasome-induced pyroptosis through inhibiting NF-κB/GSDMD signal in mice adipose tissue. *J. Pineal Res.* **63**: e12414-e12414. doi:10.1111/jpi.12414.

116. Zhang Y, Liu X, Bai X, Lin Y, Li Z, Fu J, Li M, Zhao T, Yang H, Xu R, *et al*. (2018) Melatonin prevents endothelial cell pyroptosis via regulation of long noncoding RNA MEG3/miR-223/NLRP3 axis. *J. Pineal Res.* **64** (2): doi:10.1111/jpi.12449.
117. Huang Y, Lu D, Ma W, Liu J, Ning Q, Tang F, Li L (2022) miR-223 in exosomes from bone marrow mesenchymal stem cells ameliorates rheumatoid arthritis via downregulation of NLRP3 expression in macrophages. *Mol. Immuno.l* **143**: 68-76. doi:10.1016/j.molimm.2022.01.002.
118. Liu XM, Ma L, Schekman R (2021) Selective sorting of microRNAs into exosomes by phase-separated YBX1 condensates. *Elife* **10**: e71982. doi:10.7554/eLife.71982.
119. Xu C, Wu A, Zhu H, Fang H, Xu L, Ye J, Shen J (2013) Melatonin is involved in the apoptosis and necrosis of pancreatic cancer cell line SW-1990 via modulating of Bcl-2/Bax balance. *Biomed. Pharmacother.* **67**: 133-139. doi:https://doi.org/10.1016/j.biopha.2012.10.005.
120. Kim S-H, Lee S-M (2008) Cytoprotective effects of melatonin against necrosis and apoptosis induced by ischemia/reperfusion injury in rat liver. *J. Pineal Res.* **44:** 165-171. doi:10.1111/j.1600-079X.2007.00504.x.
121. Muñoz-Casares FC, Padillo FJ, Briceño J, Collado JA, Muñoz-Castañeda JR, Ortega R, Cruz A, Túnez I, Montilla P, Pera C, *et al*. (2006) Melatonin reduces apoptosis and necrosis induced by ischemia/reperfusion injury of the pancreas. *J. Pineal Res.* **40**: 195-203. doi:10.1111/j.1600-079X.2005.00291.x.

Please cite this paper as:
Kuwabara, W., Gomes, P., Andrade-Silva, J., Soares Júnior, J., Amaral, F. and Cipolla-Neto, J. 2022. Melatonin and its ubiquitous effects on cell function and survival: A review. Melatonin Research. 5, 2 (Jun. 2022), 192-208. DOI:https://doi.org/https://doi.org/10.32794/mr112500129.