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Melatonin and sirtuins: A "not-so unexpected" relationship

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Abstract

Epigenetic modifications, including methylation or acetylation as well as post-transcriptional modifications, are mechanisms used by eukaryotic cells to increase the genome diversity in terms of differential gene expression and protein diversity. Among these modifying enzymes, sirtuins, a class III histone deacetylase (HDAC) enzymes, are of particular importance. Sirtuins regulate the cell cycle, DNA repair, cell survival, and apoptosis, thus having important roles in normal and cancer cells. Sirtuins can also regulate metabolic pathways by changing preference for glycolysis under aerobic conditions as well as glutaminolysis. These actions make sirtuins a major target in numerous physiological processes as well as in other contexts such as calorie restriction-induced anti-aging, cancer, or neurodegenerative disease. Interestingly, melatonin, a nighttime-produced indole synthesized by pineal gland and many other organs, has important cytoprotective effects in many tissues including aging, neurodegenerative diseases, immunomodulation, and cancer. The pleiotropic actions of melatonin in different physiological and pathological conditions indicate that may be basic cellular targeted for the indole. Thus, much research has focused attention on the potential mechanisms of the indole in modulating expression and/or activity of sirtuins. Numerous findings report a rise in activity, especially on SIRT1, in a diversity of cells and animal models after melatonin treatment. This contrasts, however, with data reporting an inhibitory effect of melatonin on this sirtuin in some tumor cells. This review tabulates and discusses the recent findings relating melatonin with sirtuins, particularly SIRT1 and mitochondrial SIRT3, showing the apparent dichotomy with the differential actions documented in normal and in cancer cells.

KEYWORDS

aging, apoptosis post-translational modification, cancer, deacetylases, mitochondria, SIRT1, SIRT3

1 | SIRTUINS IN THE CONTEXT OF POST-TRANSLATIONAL MODIFICATIONS (PTM)

1.1 | PTM: increasing the complexity and variability

Within the last decade, once scientist moved on from the "Human Genome Project" early in this new century, and once they realized that the not-so-many genes as expected actually exist, it became obvious that gene expression regulation has a major impact on the more than 200 different cell types in the body. Now submerged in the "omics" era, transcriptomics has become an essential discipline to understand the development and fate of a cell, that is stemness, proliferation, differentiation, or cell death. In fact, epigenetics, a term coined almost 80 years ago by Conrad Waddington even before

DNA was identified as the inheritance molecule,[1] has now reached a prominent status on biomedical sciences from multiple perspectives including health and diseases, stem cells, cloning, aging, species conservation, and evolution among others[2] and has been an expanding discipline over the past 10 years.[3,4] Several metaphors have been commonly used by experts to explain the role of epigenetics in biology including the hardware/software by Jörn Walter; he proposed DNA as the hard disk and epigenome as the software. Therefore, epigenomics, in addition to other well-known mechanisms such as alternative splicing, miRNA, or tissue-specific transcription factors, is part of the mechanisms that help to increase considerably the variability and regulatory possibilities in gene expression.[5] Hence, due to the persistence of some epigenetic markers during development, they can be potentially transmitted to offspring. These may be necessary for generating different phenotypes arising from the same genotype.[6] Furthermore, modifications and mutations in the epigenetic mechanisms can lead their involvement in many diseases including cancer.[7]

1.2 | Chemical modification of genes and proteins

1.2.1 | DNA methylation

Epigenetic modifications fall into three different categories, i.e. DNA methylation, histone modifications, and nucleosome positioning, though the interplay and the final outcome result from the interactions among them.[3,4] DNA methylation is the most widely studied of the epigenetic modification and occurs mainly in regions highly rich in the CpG dinucleotide, usually referred to as "CpG islands." They are present in many gene promoters as well as within the coding regions and generally associated with gene-silencing functions.[8] Nonetheless, other regions with a low CpG content close to the CpG islands, called "CpG island shores," have been found to be also susceptible to methylation.[9] Interestingly, these "island shores" are commonly linked to tissue specificity gene expression and important in reprogramming.[10] The DNA methyltransferase (DNMT) enzyme family is responsible for methylation, with five members described in mammals.[11] Even though melatonin actions on DNA methylation have been reported elsewhere, it is out of the scope of this review and has been already approached by other authors.[12]

1.2.2 | Histone modification

The second type of epigenetic mechanism is histone modification, a type of protein covalent post-translational modification (PTM) that comprises acetylation, methylation, phosphorylation, ubiquitylation, sumoylation, glycosylation, and ADP ribosylation. Histones play a critical role in epigenetics. The core basic histones, H2A, H2B, H3, and H4, form dimers (H2A:Hj2B and H3:H4) into a final tetramer that constitutes the nucleosome. The other histone, H1, binds to the DNA separating nucleosomes.[13] Most of the histone PTMs are reversible; that is, there are specific and separate sets of enzymes that add (writers) or remove (erasers) these marks that are essential for regulating the nucleosome stability, creating the open or closed chromatin, and ensuring, therefore, the transcriptional regulation.[14] Histone methylation is carried out by histone methyltransferases (HMT), while the corresponding opposite demethylation is achieved by the action of the histone demethylase (HDM) family. Similarly, there are sets of kinases and phosphatases that act specifically on histone subunits, although other kinases, for example, JAK2 can directly phosphorylate H3Y41. Additionally, monoubiquitination of some histones is also a reversible process which regulates both transcriptional initiation and elongation through methylation of different lysine residues.[15] Closely related to this is the sumoylation of histones, consisting in the addition of ubiquitin-like protein into lysine residues of proteins, including histones, thus antagonizing acetylation and serving as a repressive mark.[16]

1.2.3 | Histone acetylation

Acetylation, together with methylation, is a major PTM occurring on histones, and both are by far the best and most widely studied histone modifications. Acetylation (ethanoylation) is actually a common chemical addition to many organic compounds, and they are of paramount importance in protein PTMs. The process refers to the reversible modification of a lysine residue by the addition of an acetyl (CH_3CO) group, switching the positive charge of the amino acid to a neutral one and thereby changing protein function in different ways.[17] Dynamic control of core histone tail acetylation is governed by two different regulatory enzymes, that is, histone acetyltransferases (HATs) and histone deacetylases (HDACs). Consequently, antagonistic actions of these two enzyme families serve as an important mechanism for the epigenetic regulation of gene expression. Modified lysine residues are in turn specifically recognized by "bromodomain-containing proteins" which are part of complexes that modulate chromatin architecture, becoming what it is termed the "readers" for the transcription initiation.[13]

1.2.4 | Histone deacetylation

As important as acetylation during the transcription, regulatory process is the reversed action also exists. Five families of the HDACs superfamily expressed in mammalian cells are responsible for this process and include class I (HDAC1, 2, 3, and 8), class IIa (HDAC4, 5, 7, and 9), class IIb (HDAC6 and

10), class III or sirtuins (SIRT1-7), and class IV (HDAC11). Class I and class II HDACs are all Zn^{2+}-dependent deacetylases with a significant structural homology within the catalytic domain.[18,19] Class IIa regulates chromatin through the interaction with HDAC3 and shows deacetylase activity on many other targets than histones; this seems to be its primary function.[20,21] Class IIb, which includes only two members, HDAC6 and HDAC10, is unique because HDAC6 consists in two independently functioning catalytic domains and a carboxy-terminal Zn^{2+}-finger ubiquitin binding domain, while HDAC10 lacks the second catalytic domain; their exact functions are not clear.[22] Class IV enzyme, HDAC11, differs from the other two classes and is predominantly in the nucleus. It remains poorly understood, but it shows a high expression in central and peripheral nervous system and in immune system, and recently, an immunoregulatory function has been revealed.[23] Finally, class III DHACs/sirtuins (SIRTS/SIR2L) represent a structurally different family which in addition to deacetylase activity can exhibit other modifying activities such as ADP-ribosyl transferases. Table 1 shows some details regarding this classification including gene IDs, protein sizes, cofactors, subcellular localization, and tissue

TABLE 1 Types of deacetylases (HDACs) found in mammals, localization, cofactor, and tissue expression distribution

Family	Members (Gene ID)	Size (kD)	Localization	Cof.	Inhibitors[a]	Expression[b]
I	HDAC1/RPD3L1	58	Nuc	Zn^{2+}	SAHA, TSA, VPA, SB	Ubiquitous (stomach, tongue)
	HDAC2	59	Nuc			Ubiquitous (tongue, bladder, PNS)
	HDAC3/RPD3-2/SMAP45	50	Nuc/Cytop			Ubiquitous (cervix, ovary, skin)
	HDAC8/HDACL1/CDA07	44	Nuc			Ubiquitous (thymus, mammary gland, tongue)
IIa	HDAC4	120	Nuc/Cytop	Zn^{2+}	SAHA, TFMO	Ubiquitous (blood)
	HDAC5	130				Ubiquitous (thymus, BM, spleen, ovary)
	HDAC7/HDAC7A	110				Ubiquitous (liver, ovary, soft tissues, uterus)
	HDAC9/HDAC7B/HDRP	160				Ubiquitous (tongue, lymph node, larynx, BM)
IIb	HDAC6/JM21	160	Nuc/Cytop	Zn^{2+}	SAHA, TSA, VPA, SB, Tubacin	Ubiquitous (muscle, small intestine, thymus, testis)
	HDAC10	70				Ubiquitous (thymus and lymph nodes; liver, cervix)
III/Sirtuins	SIRT1/SIR2L1	120	Nuc/Cytop	NAD^{+}	Sirtinol AGK2	Ubiquitous (thymus, small int., lymph node, bone)
	SIRT2/SIR2L2	45	Nuc/Cytop			Ubiquitous (brain, muscle)
	SIRT3/SIR2L3	28	Mitoc.			Ubiquitous
	SIRT4/SIR2L4	35	Mitoc.			Only in: heart, kidney, uterus, testis, lung, liver, brain
	SIRT5/SIR2L5	36	Nuc/Cytop (Iso. 1) Mitoc (Iso. 2)			Ubiquitous (thymus, prostate, heart)
	SIRT6/SIR2L6	39	Nuc/Cytop			Ubiquitous (ovary, bone, small int.)
	SIRT7/SIR2L7	48	Nuc/Cytop			Only in skin, uterus, pancreas, ovary, brain
IV	HDAC11	39		Zn^{2+}		Ubiquitous (PNS, Brain, Testis, Prostate, Kidney)

SAHA (Vorinostat), suberoylanilidehydroxamic acid; TFMO, trifluoromethyl oxadiazole; TSA, trichostatin A; VPA, valproic acid; SB, Pracinostat, (E)-3-(2-Butyl-1-(2-(diethylamino)ethyl)-1H-benzo[d]imidazol-5-yl)-N-hydroxyacrylamide; HDAC6 selective inhibitor, tubacin, N1-[4-[(2R,4R,6S)-4-[[(4,5-diphenyl-2-oxazolyl)thio]methyl]-6-[4-(hydroxymethyl)phenyl]-1,3-dioxan-2-yl]phenyl]-N8-hydroxy-octanediamide; SIRT1 and SIRT2 selective inhibitor, sirtinol, 2-[(2-Hydroxynaphthalen-1-ylmethylene)amino]-N-(1-phenethyl) benzamide; AGK2, SIRT2 selective inhibitor, 2-cyano-3-[5-(2,5-dichlorophenyl)-2-furyl]-N-5-quinolinyl-acrylamide; PNS, peripheral nervous system; BM, bone marrow.

[a]From: Kazantsev and Thompson[19]; Mielcarek et al.[210]; Lobera et al.[211].

[b]When available, the tissues showing the maximal expression are shown. Data obtained from "Tissue-specific Gene Expression and Regulation (TiGER)" database.[24]

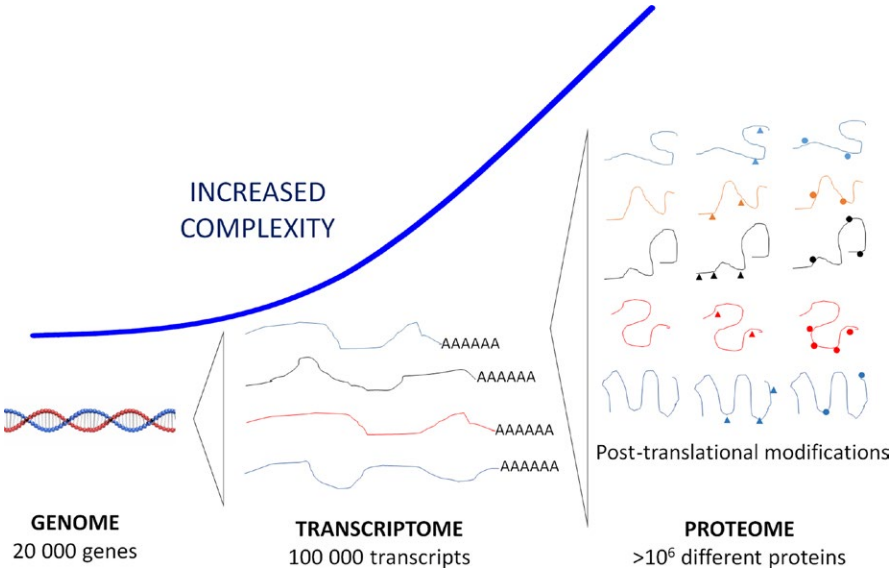

FIGURE 1 Increasing the complexity of organisms. From the 20-25 000 genes sequenced, there is an exponential increase in complexity either in the transcriptome, by alternative splicing or transcriptional modifications induced by epigenetic mechanisms and then a great variety of post-transcriptional modifications, that in fact would account for a proteome diversity, resulting in more than 10^6 different types of proteins derived from less than 5% of that number

distribution for all the members.[24,25] This review will focus only on the role of melatonin on this specific family.

Post-transcriptional modifications add also a new means of increasing variability to cells with a common gene expression pattern. However, histones are not unique as targets for acetylation, and many nonhistone proteins have been identified as the substrates of HATs and HDACs.[26] In addition to N-terminal acetylation (affecting over 80% of all eukaryotic proteins), acetylation at the ε-NH2 of lysine (ie, lysine acetylation) is a very common, reversible PTM. Furthermore, the acetylation state of numerous proteins is highly related to their stability and activity in cells as the acetyl group neutralizes the positive charge of this amino acid, changing protein function in diverse ways.[17] Much evidence suggests the importance of protein acetylation therefore increases the variability of proteins by PTM, as is illustrated in Figure 1.[27]

PTM in turn increases functional and regulatory spectra of proteins thus helping in determining cellular state and cell fate.[28,29] Consequently, modulation and alteration in protein acetylation result in abnormalities of development and physiology in different animal models.[30] Interestingly, by comparing them to bacterial acetylases, phylogenetic studies focused on the evolutionary development of mammal HDACs suggest that they preceded histone protein therefore pointing to the possibility that their truly major function is the PTM of nonhistone proteins and the action on these DNA folding proteins was actually a secondarily acquired function.[31]

Among key proteins that can illustrate the importance of acetylation, TP53 or tubulin and other microtubule-containing proteins are good examples.[32,33] There are many individual reports of acetylation sites on proteins involved in diverse biological processes, suggesting that lysine acetylation has broad regulatory functions in addition to the few that are actively studied[34] and the potential role of abnormal acetylation in different diseases has been recently considered.[35-37]

1.3 | Sirtuins

Sirtuins are class III histone deacetylase enzymes, but they are structurally and functionally different from any other HDAC family. Studies in yeasts led to the identification of some cellular factors required for transcriptional silencing, the first being the "silent information regulator 2" (Sir2).[38,39] Subsequently, other proteins encoded by the yeast SIR genes, SIR2, SIR3, and SIR4, responsible for silencing at mating-type loci, telomeres, or rDNA were found.[40] In this case, silencing was discovered to be due to particular lysine residue deacetylation in the amino-terminal tail of histones H3 and H4,[41] evidence that indicated SIR2 was a histone deacetylase; this was further confirmed in SIR2-overexpressing yeasts showing global histone deacetylation.[39] Interestingly, contrary to what occurs with SIR3 or SIR4, the SIR2 gene is highly conserved in the organisms ranging from archaea to humans. In mammals, SIR family includes seven members (see group III HDACs, Table 1) and they all show a highly conserved NAD^+-binding domain although they have different enzymatic activities and cellular functions.[42] Thus, in addition to acetylation, Frye[43] showed that SIR2 proteins can also transfer ADP-ribose group from NAD^+, observed either in bacteria, yeast, or mammals.

Sirtuins influence the cellular responses to genomic instability by regulating the cell cycle, DNA repair, cell survival, and apoptosis,[42] thus having important roles in both normal and cancer cells, including important actions on metabolic pathways by changing preference for glycolysis under aerobic conditions as well as glutaminolysis.[44] A modern overview of the hallmarks displayed by cancer cells includes loss of contact inhibition, epithelial-to-mesenchymal transition (EMT), promotion of angiogenesis, and the ability to disseminate and cause metastases in distant organs.[45] In fact, many of these features can be provided by sirtuin activation through

the regulation of cell differentiation, adhesion, cell-cell communication, and/or inflammation. Thus, sirtuins play diverse roles in cancer by affecting the response to genomic instability, regulating cancer-associated metabolism, and modifying the tumor microenvironment.

Calorie restriction (CR) is the major and most reproducible intervention for expanding life and slowing aging in different organism, from fruit flies and worms to mammals.[46] In addition to preventing age-associated decline in different parameters including the night melatonin decay,[47,48] it influences the expression of certain genes including sirtuins. These enzymes as well as NAD^+ were consequently proposed as important mediators of CR-induced benefits in lifespan in different organisms.[49-51]

1.4 | Sirtuins and metabolism

In addition to other functions, sirtuins essentially work as cellular sensors of energy availability because they consume one molecule of NAD^+ per reaction. Cellular stress caused by low-energy state increases NAD^+/NADH ratio but also decreases nicotinamide levels, activating sirtuins.[52] SIRT1 in nucleus and SIRT3 in mitochondria are the best well-known members that act as metabolic regulators.[53,54] CR improves glucose metabolism and favors mitochondrial activity by inducing the expression of SIRT1, SIRT3, and SIRT5.[55,56] CR enhances SIRT1 expression in several tissues such as brain, kidney, liver, adipose tissue, and skeletal muscle.[57] On the contrary, a high-fat diet and obesity decrease the expression of SIRT1.[58] SIRT1 not only has a role in CR but also in other diseases that drive the metabolic changes associated with diabetes, neurodegeneration, liver steatosis, bone loss, and/or inflammation.[57] Regarding their effects in cancer, sirtuins work as tumor suppressors due to suppression of the Warburg effect. SIRT3 and SIRT6 are involved in the reduction of glucose utilization toward glycolysis, while SIRT4 is related to deregulation of glutaminolysis.[59-61] However, SIRT1 is overexpressed in some types of cancer, including prostate cancer, which increases the resistance to oxidative stress,[62] while SIRT2 promotes glycolytic activity by activating phosphoglycerate mutase activity.[63] On the other hand, glucose is usually shunted to the pentose phosphate pathway in cancer cells and this is activated by SIRT2 deacetylation.[25]

One of the main metabolic targets for SIRT1 is PGC1α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha), which plays a role in gluconeogenesis and fatty acid oxidation.[64] TP53, which favors mitochondrial activity, is deacetylated by both SIRT1 and SIRT3.[53] Similarly, deacetylation of FOXO (Forkhead box) transcription factor by SIRT1 is involved in the resistance to oxidative stress or in glucose homeostasis.[53] Furthermore, SIRT1, SIRT3, and SIRT6 deacetylate and inactivate the hypoxia-inducible factor 1α (HIF1α).[65,66] In regard to mitochondrial targets, SIRT3 deacetylates mainly succinate dehydrogenase (SDH) and isocitrate dehydrogenase (IDH2),[53] as well as electron transport chain complex I promoting ATP synthesis.[67]

SIRT1 is a metabolic regulator in diabetes as it deacetylates different proteins of the insulin pathway including the insulin receptor phosphatase PTP1B (protein tyrosine phosphatase 1B) and promoting phosphorylation of insulin receptor substrates (IRSs) and PKB (phosphatidylinositol 3-kinase (PI3K)-AKT/protein kinase B).[53] As a result, SIRT1 overexpression improves the sensitivity to insulin. The feedback regulatory loop between sirtuins and metabolic regulators is also well known. Noteworthy are the cases of TP53, which represses SIRT1 transcription[68] and the major metabolic regulator, AMPKα (AMP-activated protein kinase alpha).[69] In addition, the regulation is reciprocal because SIRT1 stimulates fatty acid oxidation, thus activating AMPK.[70] As could be predicted, NAD^+ biosynthesis by NAMPT (nicotinamide phosphoribosyltransferase) is directly related to SIRT1 activity, being also an important regulator under metabolic stress.[71] At this point, AMPKα is also involved because its activation promotes NAMPT expression.[72] Mitochondrial NAD+ depletion is a major cause of cell death by genotoxic stress. To avoid that, NAMPT usually maintains mitochondrial levels by SIRT3 and SIRT4 activation.[73]

At the organism physiological levels, liver is the chief tissue where SIRT plays a critical role because it regulates glucose homeostasis in the body. PGC1α stimulates gluconeogenesis and fatty acid use,[64] while SIRT3 is critical for fatty acid oxidation.[54] In pancreatic β-cells, SIRT1 improves insulin secretion and protects these cells from hyperglycemia induced-oxidative stress.[74] In skeletal muscle, the glucose-lipid switch induced by exercise is via AMPK and SIRT1 activation and in a second step PGC1α and SIRT3.[75] In white adipose tissue, SIRT1 induces a switch to metabolically active brown fat (browning) by deacetylating the peroxisome proliferator-activated receptor (PPAR)α.[76] Finally, upon CR, SIRT1 activity also regulates vascular endothelium and hypothalamus which, respectively, promotes angiogenesis and protects from obesity and diabetes.[57] Collectively, the vast literature confirms sirtuins as key players of cell metabolism, particularly under nutrient-limiting condition. This favors mitochondrial activity at the cost of glycolysis.

2 | MELATONIN AND THE CHANGING IN ITS CONCEPTUAL VIEW

Based on the pioneering study of McCord and Allen[77] who identified frog skin lightening caused by bovine pineal extracts, melatonin, the major product of this epithalamic gland, was finally isolated and further characterized by Lerner et al.[78,79] in the late 50s. Melatonin was initially presumed to

be a factor exclusively produced in the pineal gland at night with a clear effect on reproduction, especially in terms of regulating reproduction in seasonal breeders animals by adapting their physiology to external photoperiodic conditions;[80] this is an evolutionary acquired mechanism important for ensuring survival of the offspring.[81-84] Not surprisingly, light duration and intensity modulate melatonin production by the pineal gland,[85,86] and this nocturnal production decays with age,[87,88] and this fact prompted the further investigation of the effects of melatonin on aging.[89-91]

This rather "classic" view of melatonin exclusively as a circadian modulator has been challenged, however, especially in the last 30 years, due to a number of discoveries. First, the presence of melatonin and its synthetic machinery in many tissues and organs include retina,[92] Harderian glands,[93] gut,[94] peripheral blood mononuclear cells,[95] bone marrow,[96] skin,[97] cumulus-oocyte complex,[98,99] thymus,[100] salivary gland,[101] testis,[102] and other organs of both the reproductive tract and in other systems.[103,104] In fact, the expression of melatonin synthesis rate-limiting enzymes, that is serotonin-N-acetyltransferase (known as arylalkylamine N-acetyl transferase, AANAT) and acetylserotonin-O-methyltransferase (ASMT, previously known as hydroxyindole-O-methyltransferase, HIOMT), has also been detected in most of these extrapineal sites.[105] It is believed that this wide distribution of the indole accounts for many of its functions not related to circadian adjustment.[91,106-108] In addition to its ubiquitous presence and synthesis in vertebrate organs, melatonin has been identified in evolutionary widely different organisms. Poeggeler et al.[109,110] first discovered the indole as well as its synthesis in a dinoflagellate *Lyngulodinium polyedrum* (syn *Gonyaulax polyedra*). In bacteria, its presence has been reported in alpha-proteobacteria (*Rodospirillum rubrum*)[111] and in cyanobacteria.[112] Other unicells and non-metazoan eukarya phyla in which melatonin has also been identified include Euglenoidea[113] or Kinetoplastida,[114] as well as in yeast *Sacharomyces cerevisiae*.[115] In invertebrates, it was detected in *Locusta* eyes and subsequently measured in *Carcinus maenas*, nematode *Caenorhabditis elegans*, nemertine worms, or gasteropoda among others.[116-120] In addition its presence in the in the phototrophic phyla mentioned above, it has been even discovered in fungi[120] and in vascular plants[121,122] and is also found in different phyla from red or brown algae (Rhodophyta, Phaeophyta) to green algae and land plants (Viridiplantae).[112,123-125] Overall, the presence of the indole in all organisms examined to date indicates a very ancient and basic role(s) for melatonin and supports the hypothesis that several functions of melatonin evolutionarily preceded the more "modern" circadian-related actions.[123,126]

Another milestone reached in melatonin research was achieved nearly 25 years ago when melatonin was found to show potent antioxidant activities.[127] Currently, a great deal of research relative to melatonin is focused, directly or indirectly, on oxidative stress, free radicals, or their consequences, and how the indole protects, prevents, or limits its oxidative damage.[91,128,129] Melatonin's direct scavenging leads to the formation of other metabolites which also display antioxidant activity.[130-134] This series of reaction produces a highly efficient antioxidant cascade[135] and protects multiple molecules from oxidative damage, including antioxidant enzymes themselves.[136] Indirectly, at physiological levels, melatonin increases gene expression and/or activity of antioxidant enzymes.[137-139] The pleiotropic antioxidant actions of melatonin are the underlying mechanisms for many of its important functions such as modulating apoptosis and its anti-inflammatory activities,[140-142] with or without the mediation of the G-coupled membrane receptors, namely MLNR1A and MLNR1B (formerly, MT1 and MT2, respectively). These functions and how they are related to the protective effects of melatonin have been summarized in recent reviews.[91,143]

3 | MELATONIN AND SIRTUINS

3.1 | Indirect evidence

The first report that mentions a relation between melatonin and sirtuins, namely SIRT1, was that of Das[144] more than a decade ago. He anticipated that Delta5/6 desaturases, essential for the formation of long-chain metabolites of essential fatty acids, might account for the gene expression and regulation of different factors involved in insulin resistance syndrome including melatonin and SIRT1.[144] Melatonin levels decay with age, and the nighttime reduction in serum levels has often been presumed to be contributory to the neurodegenerative diseases. Treatment of old rats for 2.5 months with melatonin not only reduced oxidative stress as expected but also concomitant increased in SIRT2 levels.[145] The same group further addressed the use of melatonin in insulin resistance as well as in senescence-accelerated prone male mice (SAMP8), finding again a preventive role of the indole with a simultaneous upregulation of SIRT1, among other genes including Pdx1 or FoxO1/3A.[146,147] A similar inverse association between melatonin treatment and aging with a simultaneous increase in SIRT1 has been reported in the dentate gyrus of rats.[148,149] Melatonin's anti-inflammatory effects as observed in an ischemic brain injury model likewise occur with the upregulation of SIRT1.[150] Collectively, these studies performed in different models document a stimulatory action of melatonin on the expression of SIRT1.

SAMP8 mice have proven to be a very valuable model to study the anti-aging properties of melatonin. These mice present age-related defects such as cognitive disability, motor dysfunction, overproduction of amyloid-β, APP, and tau, or increased amyloidosis in the brain. In contrast, control SAM/resistant 1 (SAMR1) mice age normally.[151] The accelerated age-related changes in SMP8 mice progress along with

marked signs of oxidative stress and an intrinsic activated apoptotic pathway in the brain.[152] Based on the large number of previous reports describing the antioxidant and protective properties of melatonin, oral administration of the indole protected from mitochondrial oxidative damage in these mice.[153] The oxidative damage protection was further associated to the potentiation pro-survival pathways in these mice that received melatonin in drinking water (10 mg/kg).[154] One of the most interesting features was the overexpression of SIRT1 in the mice receiving melatonin. As SIRT1 is downregulated in SAMP8 mice, melatonin via unknown mechanisms prevents this drop and restores SIRT1 values of SAM8 control levels. Thus, whether melatonin actually increased directly the SIRT1 activity or prevented collateral actions that lead to a reduction in SIRT1 activity was undetermined.

In addition to SAMP8 accelerated-aging mice studies, a role of SIRT1 in melatonin protection has also been shown in the hippocampus of normally aged mice.[155] These findings have implications for Alzheimer's disease. In this study, long-term administration of melatonin attenuated the reduction in α-secretase and inhibited the increase of β- and γ-secretases, associated with a reduction in pNFκB and a rise in SIRT1.[155]

Endoplasmic reticulum (ER) stress induced by hypoxic ischemia (HI) or by maternal inflammation in neonatal rats has been used to study the protective role of melatonin.[156,157] ER stress leads to activation of the so-called unfolded protein response (UPR), including CHOP activation.[158] Melatonin prevents CHOP expression, as well as two upstream chaperones including GRP78 and Hsp70, induced by ischemia and also preserves the SIRT1 expression, usually depleted after an injury.[156] A similar observation in CHOP and GRP78 reduction and SIRT1 upregulation has been reported in an obese murine model, focused on liver macrosteatosis. Interestingly, in this study, the authors found that the large dose of melatonin used in drinking water (100 mg/kg bw/day) increased more significantly the SIRT1 nuclear translocation, while the effects on CHOP or GRP78 were modest by comparison.[159] In this case, the authors related the rise in SIRT1 activity by melatonin with glucose metabolism rather than with ER stress. Other interesting models of inflammation with obvious therapeutic potentials include the inflammatory-induced cell death usually associated with mesenchymal stem cells engrafted into myocardial injured tissue, which limits this cell-based therapy. Interestingly, melatonin can partially rescue this pro-inflammatory-induced cell death by enhancing SIRT1 as well as decreasing acetylated forms of FoxO1, TP53, NFkB, or Bax.[160]

3.2 | Melatonin and SIRT1: a more direct approach

Some authors may question why at least some of the studies summarized above do not show a causal relationship between the indole and the sirtuins. On the whole, however, it is clear that both in vitro evidence and in vivo evidence have documented an indirect association between melatonin and sirtuins, while recent publications have provided evidence of a more obvious link between both melatonin and SIRT1 in more direct studies. Thus, Cristòfol et al. reported that melatonin increased SIRT1 expression directly in neuronal cultures from embryonic SAMP8 animals. More importantly, melatonin reduced the frailty of neurons recovered from these animals with this effect being partially abolished by the specific SIRT1 inhibitor, sirtinol, thus demonstrating an instrumental role of this sirtuin.[161] Although the partial inhibition by sirtinol could potentially support the existence of the involvement of additional mechanisms, the study clearly documents the ability to upregulate Sirt1. The SIRT1 inhibitor, sirtinol (see Table 1), has also been used to abolish melatonin protection of H2O2-mediated cell death and accelerated senescence in skin keratinocytes and in mesenchymal stem cells, respectively, unmasking the essential role of this deacetylase in melatonin's pro-autophagic actions in these cells.[162,163]

The confirmation of the fundamental role of melatonin in SIRT1 regulation was reported recently with the use of an in vivo model of severely burned rats in which the indole protected against kidney injury. In agreement with the above studies using sirtinol, the addition of other SIRT1 inhibitor (EX527) partially abolished the stimulation of SIRT1 activity and the protective effects of the indole.[164] Similarly, two in vivo models using sepsis-induced brain injury after cecal ligation and myocardial ischemia-reperfusion injury in type 2 diabetic rats both provided experimental evidence that sirtinol partially prevented the protective effects provided by melatonin treatment (20 mg/kg/day in drinking water).[165,166]

The use of SIRT1 inhibitors in all these studies points directly to SIRT1 as having a pivotal role in several functions of melatonin. The question that remains is whether the melatonin receptor-mediated function of melatonin (eg, modulation of antioxidant enzymes) explains SIRT1 modulation.

3.3 | Potential molecular mechanisms by which melatonin modulates SIRT1 activity

Much of the evidence linking melatonin to sirtuin regulation has been only recently reported, and only a few of the molecular mechanisms involved have been addressed. An explanation of direct effect of melatonin on SIRT1 was recently described by Kudová et al.[167] who found that HIF1A is essential for the melatonin-promoted cardiomyogenesis from embryonic stem cells. Although ROS-scavenging abilities of the indole could not be refuted, the melatonin membrane receptor pathway involvement was proposed to explain the process by which melatonin upregulated SIRT1 activity.[168]

In the case of the anti-inflammatory actions of melatonin on LPS-induced-oxidative stress in the rat brains, the nuclear factor-erythroid 2-related factor 2 (Nrf2) was revealed as an essential SIRT1-dependent factor, elucidated using the sirtuins inhibitor EX527.[169] Not only drug-induced inflammation but also chronoinflammation, which resulted from the age-dependent clock gene disruption, leads to a reduction in SIRT1 activity; this loss of SIRT1 activity is restored by melatonin, thus relating the indole and the deacetylase with the NLRP3 inflammasome pathway.[170] García et al.[171] had previously shown that NFKB but not NLPR3 pathway depends on RORα, an orphan receptor considered a possible nuclear binding site for melatonin. Thus, not only membrane but also a nuclear receptor may mediate the SIRT1-dependent effect of melatonin. Finally, the reduction in the acetylated forms of pro-inflammatory and pro-apoptotic proteins may explain the involvement of SIRT1 in the anti-inflammatory actions of melatonin.[160,166,168]

In a study involving peripheral blood mononuclear cells (PMBCs) from multiple sclerosis patients, SIRT1 was proposed as a final mediator of the changes in the antioxidant activity in those PMBS incubated with melatonin.[172] This again relates SIRT1 directly to the well-known action of melatonin in the modulation of antioxidant enzymes.[139]

3.4 | Melatonin and SIRT1: a dual role in cancer?

As SIRT1 is clearly involved in the aging process and considering the usual decline in nighttime melatonin levels in advance age, one consequence of this latter change may be an increased cancer risk. Jung-Hynes and Ahmad[173] were the first to propose that SIRT1 inhibition could be a molecular key for circadian clock genes gene regulation. To extent this hypothesis, in a further publication, the same group suggested a direct participation of melatonin in controlling SIRT1, but proposing the indole as an inhibitor in cancer cells in contrast to its stimulatory action on SIRT1 activity in normal cells.[174] Interestingly, SIRT1 is overexpressed in prostate cancer cells and this rise promotes cell invasion while its further inhibition halts cell proliferation via a p53-mediated pathway.[175,176]

Confirmation of this dichotomous action of melatonin regarding SIRT1 came from a more recent study using a murine model of prostate cancer, in which melatonin treatment (20 mg/L) in drinking water reduced tumor growth, proliferation parameters (eg, Ki67 or PCNA) but more interestingly caused a parallel reduction in both mRNA and protein expression of SIRT1.[177] Moreover, cell culture assays provided by Cheng et al.[178] have also shown that SIRT1 inhibition with sirtinol enhances the antitumor activity of melatonin in a human osteosarcoma model, confirming previous data. This report proves that SIRT1 is a critical regulator for apoptosis, but does not necessarily identify the deacetylase as a direct target of melatonin. Other factors such as BMAL1 clock gene might account for this regulation of Sirt1.[179]

The collective findings led one group of workers to propose the attractive theory that SIRT1 is the major reason melatonin is pro-apoptotic in cancer cells, but not in normal cells.[180] According to this theory, the actions of melatonin are context specific. In agreement with this idea, Proietti et al.[181] have found that levels of p53 acetylation and the ubiquitin protein ligase, MDM2, are drastically reduced or downregulated, whereas those of p300, on the contrary, are overexpressed in MCF-7 breast cancer cells incubated with melatonin. These responses appear to be directly related to a simultaneous reduction in SIRT1, a specific p300 inhibitor.[181] This is further evidence supporting the idea that Sirt1 inhibition by melatonin may have a direct impact in tumor growth inhibition. Although these studies do not provide unequivocal support for the idea for the role of sirtuin inhibition in anticancer actions of melatonin, they again point to sirtuins as potential targets of melatonin. A contradictory finding has been published in a recent study performed in nonsmall cell lung cancer (NSCLC) which demonstrated that the elimination of "cell cycle and apoptosis regulator 2 factor" (CCAR2) is essential to sensitize cells to melatonin-induced apoptosis; this cell death is reportedly not dependent on SIRT1.[182]

The inhibition of prostate and breast cancer cells by melatonin has been a consistent finding, and the actions of melatonin on lung cancer cell inhibition is consistent with these results.[183] As an interaction with sirtuins may be necessary for melatonin's inhibition of cancer, more research should be directed to this relationship. It is also becoming clear that melatonin's action depends on whether the cells or which it is acting are normal or cancerous, that is, melatonin's actions are context specific.[180] This specificity of action may involve the ability of melatonin to stimulate/inhibit SIRT1 activity. The interplay of other protein mediators, that is, clock genes, might also account for the differential actions of melatonin on SIRT1 activity.

With regard to clock genes, because SIRT1 is a well-known circadian clock regulator, some suggested that melatonin-induced SIRT1-inhibition in tumor cells might be of importance not only in terms of proliferation exclusively but also for resynchronizing clock genes. Clock genes such as *Bmal1* are key players in controlling tissue homeostasis and crosstalk with SIRT1 possibly being an essential key for this regulation that could involve feedback regulatory loop.[179,184-186]

In conclusion, however, a dual role for melatonin in regulating Sirt1 and clock genes in normal and cancer cells cannot be ruled out even though the data obtained in cancer cells studies are not always consistent with melatonin causing reductions of both SIRT1 and tumor development. Moreover, a recent study using both melatonin and resveratrol as SIRT1 enhancers in a porcine oocyte maturation model has clearly

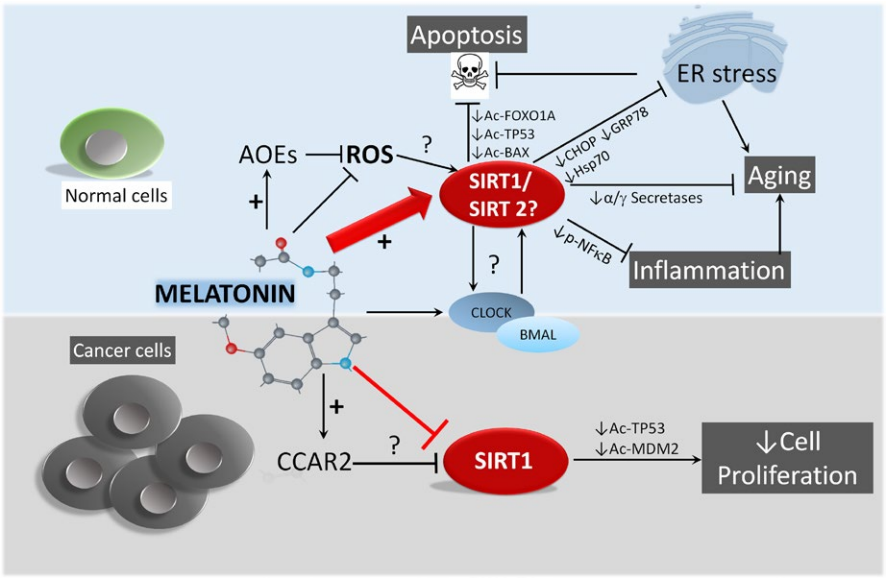

FIGURE 2 An overview of the actions of melatonin on SIRT1/2 reported to date. Melatonin seems to act differently in normal (top panel) or in cancer cells (bottom panel). While melatonin increases SIRT1—and possibly SIRT2—activity in a normal physiological context, several reports have confirmed the inhibitory effect on SIRT1 in cancer cells, which alters completely the biological outcome. Melatonin increases activity and/or gene expression of AOEs as well as scavenge directly ROS, and these actions might underlie the SIRT1/2 activating effects. However, direct effects on SIRT1/2 (red arrow) cannot be ruled out. This activation would in turn reduce apoptosis by reducing the acetylated levels of FOXO1A, TP53, or BAX. Additionally, SIRT1 activation has also an impact in ER stress by reducing CHOP, GRP78, or Hsp70 levels. Other targets of SIRT1 are related to the inflammatory process, including phosphorylated NFκB, which would account for the anti-inflammatory effects exerted by this deacetylase. Finally, a direct inhibition on alpha and gamma secretases reduces the levels of β-amyloid protein and would slow the aging process. In cancer cells, melatonin blocks SIRT1, usually highly activated in tumors, which in turn would reduce acetylated forms of TP53 or MDM2 and as a consequence would reduce cell proliferation. Abbreviations used are as follows: AOEs, antioxidant enzymes; ROS, reactive oxygen species; Ac-FOXO1A, acetylated form of Forkhead box protein 01; Ac-TP53, acetylated form of p53; Ac-BAX, acetylated form of apoptosis regulator Bax; ER, endoplasmic reticulum; CHOP, DNA damage-inducible transcript 3; GPR78, G protein-coupled receptor 78; Hsp70, heat-shock protein 70 kD; p-NFKB, phosphorylated form of nuclear factor kappa B; CCAR, cell cycle and apoptosis regulator 2; Ac-MDM2, acetylated form of E3 ubiquitin protein ligase Mdm2

shown that SIRT1 may not be a target of the indole, because melatonin potentiated the beneficial effects of the stilbene when they were combined.[187] Thus, whether clock genes are major players in the melatonin-mediated regulation of sirtuins or, on the contrary, SIRT1 and/or other sirtuins are instrumental in clock gene modulation by melatonin deserves further attention. It should be noted that SIRT1 can form a complex with CLOCK/BMAL dimer as well as deacetylate PER2, among other potential regulatory pathways.[188,189] An overview of the effects documented to date is depicted in Figure 2.

3.5 | Melatonin and other sirtuins: the special case of SIRT3

SIRT1 has been the most widely investigated sirtuin in reference to melatonin, especially in models of aging and inflammation. SIRT2 also has, however, received attention. In the study of Kireev et al.[148] that focused on the neuronal apoptosis in the dentate gyrus from aged mice, the authors reported that SIRT2 was not changed as a consequence of melatonin treatment. This, however, is opposite to the findings where melatonin treatment reduced SIRT2 activity in the colon of aged rats.[190] In this report, melatonin did provide protection against oxidative stress associated with the aging process.

More interesting is the possible role of melatonin on the expression and/or activity of specific mitochondrial sirtuins, particularly SIRT3. Mitochondria appear to be major targets for melatonin, and many functions of the indole are somehow related to mitochondrial function.[91] There is an extensive literature focused on this topic which is out of the scope of this review.[191-193] Mitochondria—and chloroplasts—have even been proposed as the original sites for melatonin synthesis.,[194] and melatonin concentrations in mitochondria seem to be exceptionally high.[195] The ability of melatonin to influence the activities of mitochondrial sirtuins represents an unexploited research field that may prove interesting.

SIRT3 is located primarily in the mitochondrial matrix where regulates most of the lysine acetylation that takes part within this organelle.[54] Due to its location, it is involved in a variety of mitochondrial processes including fatty acid and acetyl-CoA metabolism, apoptosis, electron transport chain function, and endogenous antioxidant defenses.[196,197]

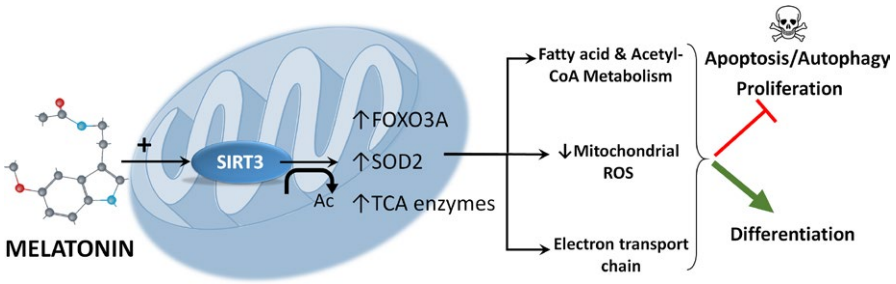

FIGURE 3 A diagram depicting the documented findings relating melatonin and mitochondrial SIRT3. Within mitochondria, melatonin can stimulate SIRT3 activity which deacetylates, among others, FOXO3A, SOD2, or most of the metabolic enzymes involved in the TCA cycle. As a result, SIRT3 activation inhibits cytochrome c release and apoptosome activation, and autophagy. Additionally, a higher SOD2 activity by deacetylation and the metabolic shift in mitochondria caused by the mitochondrial sirtuin can lead to a reduced cell proliferation rate and a higher differentiation state. Abbreviations used are as follows: ROS, reactive oxygen species; FOXO3A, Fork head box protein 3; SOD2, superoxide dismutase 2; TCA, tricarboxylic acids cycle (Krebs)

Accordingly, SIRT3 has also been associated with age-related illnesses such as insulin resistance, fatty liver, and cardiac hypertrophy.[198,199] Furthermore, this enzyme may function as a tumor suppressor because knockout mice spontaneously develop estrogen and progesterone-positive well-differentiated mammary tumors which are also age-related.[200]

The role that SIRT3 plays in mitochondrial antioxidant defense appears to be essential. Mitochondrial superoxide dismutase, SOD2, is an important antioxidant enzyme and $SOD2^{-/-}$ mice exhibit neonatal lethality, neurodegeneration, and cardiomyopathy.[201] Thus, SOD2 is activated by SIRT3-mediated deacetylation of lysine 122, 68, 53, and 89 which alters its enzymatic activity. SIRT3 also interacts with FOXO3a thereby increasing the transcription of SOD2 and catalase.[197,202] SOD2 is furthermore implicated in cellular differentiation. Thus, an upregulation of SOD2

TABLE 2 An overview of the results obtained with melatonin and sirtuins

Sirtuin	Model	Effect of melatonin	Effect on Sirtuin	Use inhibitors to show direct role	Refs.
SIRT1	SAMP8 mice	Slows senescence, reduce mitochondrial oxidative damage	+	No	147,151-154
	Aged rats	Slows aging parameters	+	No	148,149
	Ischemic brain injury	Anti-inflammatory	+	No	150
	Aged normal mice	↑α-secretase; ↓β/γ-secretase; prevents pNFkB in hippocampus	+	No	155
	ER stress	Prevents ↑CHOP, ↑GRP78, ↑Hsp70	+	No	156-158
	Mesenchymal SCs	Prevents Inflammation-induced cell death	+	No	160
	Primary neuronal cultures (E17)	Prevents cell death	+	Yes	161
	H2O2-induced toxicity	Prevents cell death	+	Yes	162,163
	Skin keratinocytes	Avoids senescence	+	Yes	162
	Rat	Protects from kidney injury	+	Yes	164
	Rat	Prevents brain injury Prevents myocardial damage	+	Yes	165-166
	Prostate cancer cells and TRAMP mice	Antiproliferation, tumor growth inhibition	−	No	177
	MCF-7	↓ acetylated TP53 and MDM2	−	No	181
	Nonsmall cell lung cancer (NSCL)	Induction of apoptosis	−	No	183
SIRT2	Aged rats	Reduction in oxidative stress	+	No	148
	Aged rats	Prevent colon aging	+	No	190
SIRT3	Cultured hepatocytes	Cadmium-induced mitochondrial toxicity; Oxidative damage	+	Yes	207-208

produces neuroendocrine differentiation in prostate cancer cells.[203] Similarly, an induction of SIRT3 and SOD2 by RANKL diminishes intracellular ROS and negatively regulates osteoclast differentiation.[204] Moreover, TGFβ1 reduces the expression of both SIRT3 and SOD2 in lung fibroblasts promoting myofibroblast differentiation.[205] Consequently, SIRT3 seems to mediate cell fate by regulating SOD2 among other factors. Other antioxidants and sirtuin activators such as resveratrol regulate both protein levels and SIRT3 enzymatic activity.[206]

While the role of melatonin on SIRT3 activity has not been well investigated, the work of Pi et al.[207] provides a proof-of-concept study regarding the likely involvement of the mitochondrial sirtuin in the hepatoprotective effect of melatonin in cadmium-induced toxicity.[207] Cadmium induces mitochondrial-related, superoxide anion-induced autophagic cell death, with a simultaneous reduction in SIRT3. The specific inhibition of SIRT3 with 3-(1H-1,2,3-triazol-4-yl)pyridine blocks melatonin's preventive effect, thus showing that SIRT3 is a key factor in melatonin's critical importance of the sirtuin in the indole antioxidant action.[207] A more recent study performed by Chen et al.[208] in hepatocytes corroborated the previous work, showing that SIRT3 mediates the antioxidant actions of the melatonin in hepatocytes. The essential role of the sirtuin in this protection was confirmed using siRNA against SIRT3, which blocked melatonin's actions.[208]

In both studies summarized above, a major target of SIRT3-mediated action was the reduced acetylated levels of the essential mitochondrial antioxidant enzyme, SOD2. Melatonin increases expression and/or activity of antioxidant enzymes,[139] particularly SOD2, which is involved in the indole-mediated differentiation in some cell types.[209] SOD2 also plays a role as a protective agent in neurodegenerative disorders. As a result, the aforementioned experiments indicate that many of the indirect antioxidant actions exerted throughout the stimulation of antioxidant enzymes could be easily explained with the direct stimulation of gene and/or activity of SIRT3. Additional reports will likely shed more light on this essential issue because the mitochondria are the key organelles in many diseases and appear to be a major site for melatonin's actions. Figure 3 summarizes the evidences found relating melatonin and SOD2.

4 | CONCLUDING REMARKS

As the discovery of the antioxidant, immunomodulatory, oncostatic, and neuroprotective actions of melatonin decades ago, many researchers feel that some common yet-to-be-identified mechanism(s) may mediate the widespread actions of this indole.[91] This basic action may involve membrane or nuclear receptors or receptor-independent processes. The actions reported to date on SIRT1 and to a lesser extent on SIRT3, which are summarized in Table 2, indicate these deacetylases may be key players that could explain many of the functions of melatonin. Additional mechanistic studies are required to confirm the involvement of these deacetylases in a variety of cell types (normal and cancer cells). Considering that both melatonin and sirtuins have a long evolutionary history, it would be surprising if they do not somehow interact.

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