Trends in Endocrinology & Metabolism

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Review

Functional Interaction between Melatonin Signaling and Noncoding RNAs

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Melatonin was discovered in the pineal gland and first came to be known as a biochemical synchronizer of circadian rhythm. The molecular mechanisms underlying the broad-spectrum actions of melatonin are not restricted to its interaction with proteins but it also has functional effects after influencing RNA species that have no protein-coding potential. In this review we discuss the current understanding of the melatonin-mediated modulation of noncoding RNA (ncRNA) pathways under different physiological and pathological conditions. We also delineate the impact of specific ncRNAs in controlling the synthesis of melatonin. The information compiled herein will serve as a solid foundation to formulate ideas for future mechanistic studies on melatonin and to better explore the emerging functions of the noncoding transcriptome.

Highlights

Melatonin is a multitasking molecule that modulates diverse functions in many organs and cell types. This involves the regulation of gene expression in myriad developmental/pathological stages and conditions via the coordination of transcription factors and ncRNAs.

Melatonin, through functional interactions with specific miRNA pathways, exerts its regulatory effects on the development of cancer, brain conditions, liver fibrosis, ASD, atherogenesis, and spermatogenesis in different species.

Recent whole-transcriptome analyses have greatly extended our knowledge on the biology of lncRNAs, another class of remarkably numerous and functionally versatile ncRNAs. Functional involvement of specific lncRNAs in oncostatic actions of melatonin has now been recognized.

Introduction

ncRNA

In light of the recognized importance of protein molecules in mediating biological functions, RNA was considered originally as merely an intermediate molecule for passing the genetic code from DNA to final protein products, as proposed in the central dogma of molecular biology. This notion was later challenged when an enormous number of ncRNAs were discovered and their significance became increasingly clear (Table 1). Recent advances in sequencing technologies have revealed that at least 70% of the human genome is transcribed into RNA while only less than 2% encodes proteins [1,2]. This has resulted in a paradigm shift in our understanding of the potential impact of the noncoding **transcriptome** (see Glossary) [3]. Some of the ncRNAs are constitutively expressed in all cells and essential for fundamental aspects of cell biology, thus being known as housekeeping ncRNAs. Housekeeping ncRNAs include small-sized transcripts such as tRNA for carrying amino acids, small nuclear RNA (snRNA) for RNA splicing, and small nucleolus RNA (snoRNA) for RNA modification as well as large-sized transcripts, such as rRNA for protein translation.

In addition to the housekeeping ncRNAs, another functionally distinct category of ncRNAs whose primary function is to regulate gene expression is referred to as regulatory ncRNAs. Based on an arbitrary length cutoff of 200 nucleotides, regulatory ncRNAs are roughly divided into small ncRNAs and long ncRNAs (lncRNAs). Depending on the size, biogenesis, action, and targets with which they are associated, small ncRNAs can be further classified into several subgroups including miRNAs, endogenous siRNAs (endo-siRNAs), P element-induced wimpy testis (PIWI)-interacting RNAs (piRNAs), and many others [4]. miRNAs, endo-siRNAs, and piRNAs often employ their sequences in a complementary manner to silence the expression of their target genes. Nevertheless, lncRNAs can act through genomic targeting, regulation in cis (affecting adjacent genes) or trans (affecting distant genes on different chromosomes), epigenetic mechanisms, and antisense interference to up- or down-regulate gene expression due to not only primary sequence similarity but also the formation of

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Table 1. Classes of Human ncRNAs and Their Sizes and Functions

Abbreviation	Full name	Size[a]	Function
Housekeeping ncRNA			
rRNA	Ribosomal RNA	120, 160, 1868, 5025 nt	Translation machinery
tRNA	Transfer RNA	70–90 nt	Carrier of amino acid
snRNA	Small nuclear RNA	100–300 nt	RNA processing
snoRNA	Small nucleolar RNA	70 nt	RNA modification
TERC	Telomerase RNA component	450 nt	Telomere synthesis
Regulatory ncRNA			
miRNA	MicroRNA	21 nt	RNA stability and translational regulation
endo-siRNA	Endogenous small interfering RNA	22 nt	RNA degradation
piRNA	PIWI-interacting RNA	27 nt	RNA decay and transposon silencing
lncRNA	Long noncoding RNA	>200 nt	Genomic imprinting, epigenetics, scaffold, regulation of gene expression

[a]nt, nucleotide.

Glossary

CRISPR–Cas9 screening: a method using the bacterial CRISPR–Cas9 system for the knockout of individual genes in functional screens. By delivering the CRISPR-associated nuclease Cas9 complexed with a RNA that guides the system to matching sequences of DNA in a cell, the genomic sequence can be cut at a desired location.

Transcriptome: the entire set of all RNA molecules expressed in a cell or a population of cells.

Transposon: a sequence of DNA that can move from one genomic location to another by a copy–paste mechanism. The transposition often alters the genetic identity and genome size of a cell, although most transposons are no longer active.

secondary structures [5]. Unlike the housekeeping ncRNAs, the expression of these small ncRNAs and lncRNAs is highly regulated in different cell types or at different stages during development. Given the lengthy list of novel ncRNA classes and their extensive regulatory roles, for a discussion of a complete repertoire of the ncRNA transcriptome and their functions the reader is directed to other reviews [3,4].

Melatonin: A Pleiotropic Regulatory Hormone

Melatonin (*N*-acetyl-5-methoxytrypamine), originally uncovered as a neurohormone of the bovine pineal grand, is generated in a multitude of tissues and cell types [6]. In the pineal gland, its synthesis and release are orchestrated by light–dark cycle conditions, whereby daylight lessens and darkness leverages melatonin levels. In addition to its recognized effect on regulation of the phasing of circadian rhythms and sleep promotion [7], this multifaceted agent is a substantial immunomodulatory molecule [8] but also a powerful free radical scavenger [9]. Melatonin, in part, functions through the G protein-coupled receptors melatonin receptor 1A (MT1) (encoded by MTNR1A) and MT2 (encoded by MTNR1B), expressed throughout the central nervous system (CNS) and perhaps in all peripheral tissues to regulate some of its biological activities under various physiological and pathological conditions [7,10].

With the recent explosion of vital contributions of the noncoding transcriptome, earlier knowledge focused on activation or repression of transcription factors as well as on interactions with nucleosomes to be remodeled during gene activation is insufficient in capturing the entire picture of gene regulation by melatonin signaling. In recent years a large body of evidence has revealed comprehensive involvement of melatonin in ncRNA biology. In this review we cover current advances in our understanding of melatonin-mediated modulation of ncRNA pathways in different circumstances. We also discuss the impact of specific ncRNAs on the control of the synthesis of melatonin or its receptors. The information compiled herein will serve as a solid foundation to formulate ideas for future mechanistic studies on melatonin.

miRNA

miRNAs, a large family of post-transcriptional regulators of gene expression that are ~21 nucleotides in length, are among the most extensively studied of all categories of ncRNAs that are functionally related to melatonin signaling. Extensive discussion on the details of miRNA biogenesis and function can be found elsewhere [11,12]. Here, the involvement of melatonin-mediated miRNA pathways in various physiological and pathological conditions (Figure 1) in different species is discussed below.

Melatonin-Mediated Coordination of miRNA Pathways in Malignancies

Melatonin under both *in vitro* and *in vivo* conditions is known to inhibit the growth of various cancer types as well as tumor metastases [13,14]. These versatile mechanisms underlying melatonin's actions to antagonize tumor growth and invasion involve differential expression of protein-coding genes and a unique miRNA signature triggered by melatonin signaling.

A panel of 22 specific miRNAs, together with their putative protein-coding target genes, has been previously found to be regulated on melatonin-mediated suppression of breast cancer cell proliferation [15]. In this study these downstream protein-coding genes of selected melatonin-regulated miRNAs are functionally linked to negative regulation of the mitotic cell cycle, phospholipid translocation, regulation of the lipid biosynthetic process, and negative chemotaxis, providing an insight into a role of miRNAs in the short-term anticancer effects of melatonin. Moreover, manipulation of the expression of two regulated miRNAs, miR-363-3p and miR-1207-3p, further

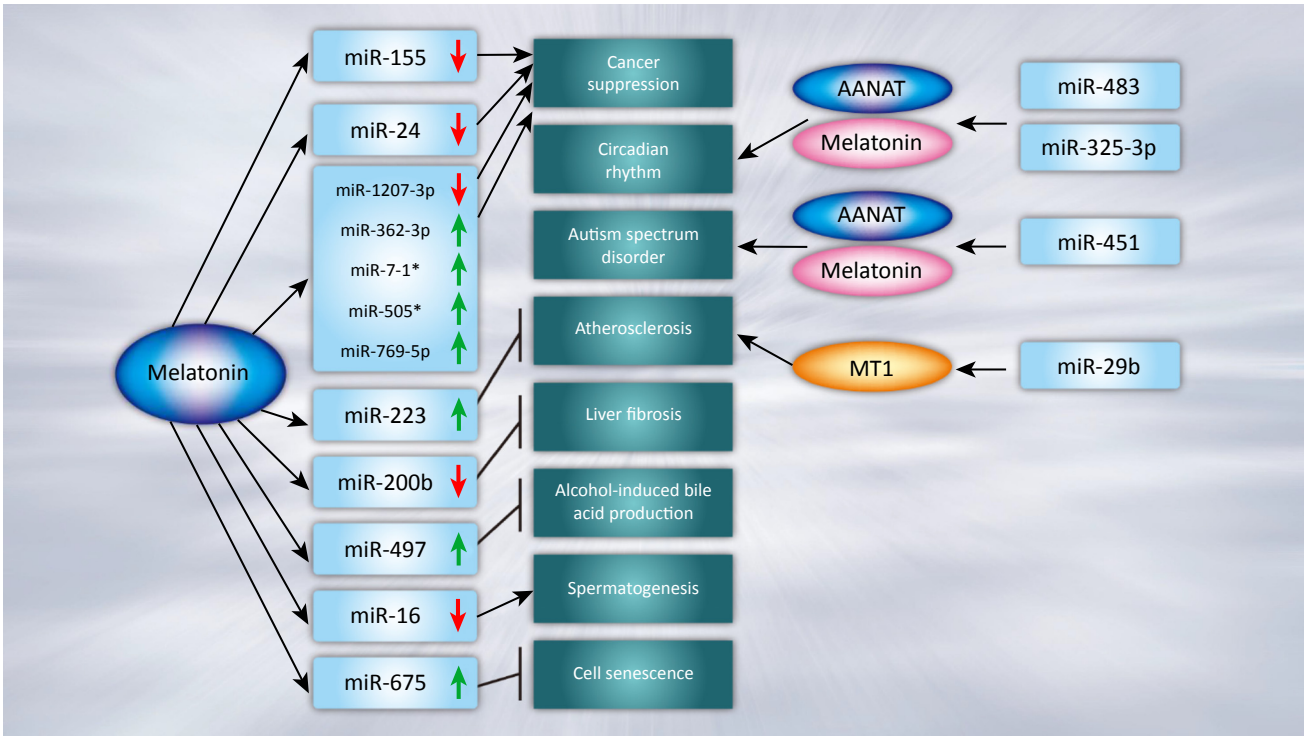

Figure 1. Functional Interactions between miRNAs and Melatonin Signaling. The red and green arrows represent downregulation and upregulation, respectively. The black lines, arrows, and bar-headed lines represent the association, promotion, and inhibition of a gene, a pathway, or a condition, respectively. The diagram is based on the findings from various studies cited appropriately in the text. AANAT, aralkylamine *N*-acetyltransferase; MT1, melatonin receptor 1A.

verified the functional relevance of miRNAs in melatonin-mediated expression of cancer-related genes. Intriguingly, another investigation that focused on the long-term anticancer effects of melatonin in breast cancer cells identified that miR-24 was downregulated post-transcriptionally through a reduction in the hnRNP A1 level mediated by melatonin treatment for 72 h [16]. Overexpression of miR-24 promoted p38- and p53-dependent DNA damage in breast cancer cells, indicating an oncostatic role of melatonin in preventing cells from accumulation of DNA damage through suppression of miR-24. Findings from these two reports suggest that different concentrations and durations of melatonin treatment have distinct impacts on miRNA expression, which may be linked to the various anticancer properties of melatonin in breast cancer biology.

Melatonin has also been shown to contribute to the growth inhibition of glioma cells [17] and enhance the efficacy of oncostatic compounds in glioblastoma through epigenetically regulating the expression of an ABC transporter gene, ABCG2 [18]. Recently, a connection between an oncogenic miRNA, miR-155, and antiglioma mechanisms by melatonin was elucidated in an investigation where melatonin was shown to downregulate miR-155 to counteract proliferation and invasion of glioma cells via repression of a transcriptional activator, c-MYB [19]. These results reveal intricate regulation of not only protein-coding genes but also miRNAs by melatonin and provide potential avenues for the use of melatonin as a complementary anticancer treatment.

Functional Interaction between Melatonin Signaling and miRNA Pathways in Brain Conditions

Since the pineal gland is an important melatonin-producing organ, it is conceivable that numerous melatonin functions are relevant to brain physiology and pathology. Notably, miR-483 was downregulated dramatically during ontogeny and found to directly target the mRNA for aralkylamine *N*-acetyltransferase (AANAT), the key rate-limiting enzyme of melatonin formation in the rat pineal gland [20]. Moreover, transfection of neonatal pinealocytes with a miR-483 antagonist resulted in a marked increase in melatonin synthesis, indicating that miR-483 may contribute to low melatonin formation in the pineal gland of neonates. Other than the stages during development, another miRNA, miR-325-3p, was also identified to suppress melatonin production by interacting with the 3′ untranslated region (UTR) of AANAT mRNA after hypoxic–ischemic brain damage [21]. Such a relationship between miR-325-3p and melatonin levels provides additional clues to the pathological mechanism underlying impaired circadian rhythms after neonatal brain damage.

In addition to a role in hypoxic–ischemic brain injury, melatonin possesses anti-inflammatory properties in the CNS and other peripheral tissues [22,23]. As a potent immune modulator, melatonin reverses lipopolysaccharide (LPS)-induced brain inflammation through regulation of endoplasmic reticulum (ER) stress, autophagy, and the miR-34a–silent information regulator 1 (SIRT1) pathway [24]. miR-34a has been functionally implicated in ER stress [25], autophagy [26], and inhibition of LPS-induced inflammatory responses in murine macrophages [27]. Evidence from these investigations suggests that melatonin exerts its immunomodulatory and neuroprotective actions by coordinating crosstalk between miRNAs and these interrelated pathways.

Melatonin-Regulated miRNAs in Liver Fibrosis

Several lines of evidence have indicated that melatonin exhibits protective effects on a variety of liver diseases, including cholestatic liver injury, alcohol-induced liver injury, nonalcoholic fatty liver disease, liver fibrosis, cirrhosis, and hepatocarcinoma [28]. It is reported that melatonin administration showed therapeutic effects on hepatic fibrosis in a genetically

engineered mouse model of sclerosing cholangitis ($Mdr2^{-/-}$) through downregulation of miR-200b [29]. The level of miR-200b was elevated in $Mdr2^{-/-}$ mice and patients with sclerosing cholangitis whereas it was reduced in $Mdr2^{-/-}$ mice subjected to melatonin treatment [29]. Overexpression of AANAT or silencing of miR-200b lowered the expression of miR-200b and fibrotic genes in cholangiocytes and hepatic stellate cells. These data suggest that targeting the melatonin–miR-200b axis may be helpful in the management of biliary damage and liver fibrosis in cholangiopathies. Similarly, in a study focusing on hepatic metabolic dysfunction caused by alcohol, another melatonin-regulated miRNA, miR-497, may ameliorate alcohol-induced excess of hepatic bile acid via targeting a major driver of hepatic metabolic diseases, cannabinoid receptor type 1 (CB1R) [30]. Collectively, specific melatonin–miRNA pathways may serve as targets for novel therapeutic strategies against hepatic damage and disease.

Association of Melatonin–miRNA Axis with Autism Spectrum Disorders (ASDs)

ASD is a neurological and developmental disorder characterized by difficulty in social communication, presence of stereotypy, and limited interests and activities. In addition to its highly heterogeneous genetic basis [31], several biochemical imbalances have been observed in patients with ASD, including neurochemical, immunological, or metabolic traits [32,33]. Among these, a reduction in melatonin levels in either the urine or the circulation of ASD patients has been detected in several reports [34–36]. In a recent study, the deficit in pineal melatonin levels of ASD patients was proposed to be derived from a post-translational and a post-transcriptional effect on the suppression of AANAT activities by 14-3-3 protein and miR-451, respectively [37]. Although genomic factors seem to be in part implicated in such an impairment of melatonin synthesis, as genetic variants that lead to reduction of AANAT activities were found in a small proportion of ASD patients, the connection among miR-451, 14-3-3 protein, and melatonin production has reshaped our understanding of the complexity of autism pathophysiology.

Melatonin Signaling and miRNA in Atherosclerosis

Atherosclerosis is a multifactorial process that is often initiated with endothelial dysfunction and apoptosis [38]. A high-fat diet (HFD) that increases endothelial permeability and apoptosis [39] is associated with the development of atherosclerosis [40]. In an investigation using a genetic mouse model of atherosclerosis ($apoE^{-/-}$), one specific miRNA, miR-29b, was induced by HFD and shown to enhance endothelial permeability and apoptosis in HFD-stimulated mice [41]. Further analysis revealed that miR-29b targeted and inhibited the expression of MT1 through a binding site located in the 3′-UTR of MT1 mRNA. Melatonin is considered a cardioprotective agent as it attenuates molecular and cellular damage resulting from cardiac ischemia–reperfusion [42]. These observations verify MT1 as a direct target of miR-29b and demonstrate an effect of miR-29b-regulated melatonin signaling in governing endothelial permeability and arterial apoptosis in cardiovascular diseases.

Involvement of miRNA Pathways in Melatonin-Regulated Spermatogenesis

Melatonin's action is also important for spermatogenesis and animal reproduction [43]. Administration of melatonin improves the testicular development and semen quality of rams and male Damascus goats during the nonbreeding season [44,45]. The expression profile of miRNAs in the testes of melatonin-treated mice was previously defined [46]. Among differentially expressed miRNAs, miR-16 was downregulated while melatonin treatment promoted cell growth and reduced apoptosis in mouse-derived spermatogonia (GC-1 spg) cells. Manipulation of miR-16 expression further verified its functional relevance in GC-1 spg cells. Moreover, bioinformatics analysis and reporter assays revealed that cyclin D1 (CCND1) is a potential target

for miR-16. These results suggest that peripheral melatonin acts as a regulator of testicular function by modulating specific miRNA signatures.

lncRNA

lncRNAs are generally defined as RNA transcripts longer than 200 nucleotides with no protein-coding role. For the details of lncRNA biogenesis and function, the reader is directed to other excellent reviews [47–49]. In a previous study aiming to explore circadian changes in lncRNA expression, the repertoire of lncRNAs with either daily changes in abundance in the rat pineal gland or high relative expression compared with a pool of RNA from other tissues was defined by RNA sequencing, indicating a dynamic role of lncRNAs in vertebrate circadian biology [50]. In addition, accumulating evidence has pointed to a functional interaction between melatonin signaling and lncRNA pathways. These melatonin–lncRNA relationships in many developmental or pathological processes are summarized below (Figure 2).

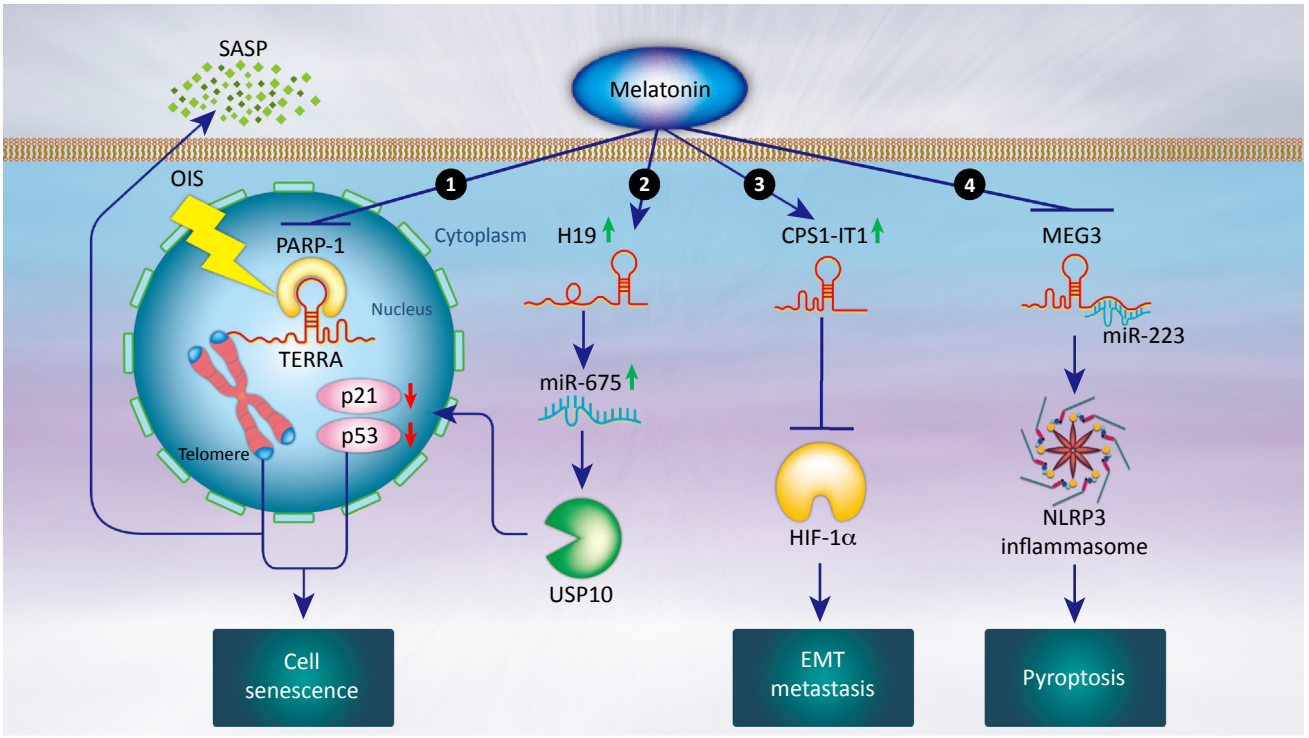

Figure 2. Schematic Diagram of Melatonin-Mediated Long Noncoding RNA (lncRNA) Pathways in Cell Senescence, Hepatic Cancer Suppression, and Endothelial Cell Pyroptosis. (1) In human fetal lung fibroblast cells, oncogene-induced senescence (OIS), an antiproliferative response triggered by accumulated genomic alterations on oncogenes, is frequently accompanied by the secretion of an intricate mixture of factors, which is known as the senescence-associated secretory phenotype (SASP). Melatonin treatment antagonizes the interaction between the lncRNA telomeric repeat-containing RNA (TERRA) and poly(ADP-ribose) polymerase 1 (PARP-1), further maintaining genome integrity and attenuating the SASP on OIS. (2) In addition to differentiated cells, C-kit$^+$ cardiac progenitor cells (CPCs) may undergo cell senescence in response to oxidative stress. As oxidative stress causes downregulation of the lncRNA H19 and an miRNA derived from H19, miR-675, melatonin treatment replenishes the levels of H19 and miR-675 in CPCs. Further, miR-675 targets USP10 mRNA, which in turn results in the downregulation of two regulators of the cell cycle, the p53 and p21 proteins, ultimately leading to cell senescence. (3) The hypoxia-inducible factor 1 alpha (HIF-1α) pathway is a key regulator of hepatic cancer progression. Melatonin treatment induces the expression of the lncRNA CPS1 intronic transcript 1 (CPS1-IT1), which attenuates HIF-1α activity and subsequently leads to the suppression of the epithelial–mesenchymal transition (EMT) and liver cancer metastasis. (4) Pyroptosis is a highly inflammatory form of programmed cell death during which the NLRP3 inflammasome is activated to mediate the processing and secretion of proinflammatory cytokines (not shown here). In pyroptotic endothelium, melatonin induces the lncRNA MEG3, which acts as an endogenous sponge by sequestering miR-223. Suppression of miR-223 function by melatonin-regulated MEG3 elevates the expression of many inflammasome-related genes, thereby inducing endothelial cell pyroptosis.

Melatonin-Regulated lncRNA Pathways in Cell Senescence

Cardiac progenitor cells (CPCs) have demonstrated potent regenerative ability in ischemic and infarcted myocardium after transplantation; as a result, they have emerged as an appealing tool for the treatment of heart diseases [51]. Nevertheless, transplanted CPCs may be unable to proliferate and differentiate due to cellular senescence induced by local pathological stimuli such as oxidative stress [52]. As a pleiotropic regulatory agent, melatonin has been shown to modulate many biological behaviors of stem cells in numerous investigations [53–57]. Among these studies, one has revealed that melatonin antagonizes premature senescence of CPCs in response to oxidative stress [57]. Interestingly, in this study a well-annotated lncRNA, H19, and a miRNA derived from H19, miR-675, were downregulated by H_2O_2 in CPCs, with their expression levels being replenished by melatonin treatment. Silencing of H19 or miR-675 counteracted melatonin-mediated inhibition of premature senescence in CPCs exposed to H_2O_2. Further analysis showed that H19-derived miR-675 targeted to the 3′-UTR of USP10 mRNA, which in turn resulted in the downregulation of two regulators of the cell cycle, the p53 and p21 proteins. These data provide novel insights into the functional involvement of specific lncRNAs in the pharmacological actions of melatonin on CPCs.

Other than stem and progenitor cells, senescence of differentiated cells is accompanied by the secretion of an intricate mixture of factors, which is known as the senescence-associated secretory phenotype (SASP) [58]. Paradoxically, acquisition of a SASP has deleterious effects on the tissue microenvironment and, surprisingly, favors cancer development [59]. In a recent study [60], melatonin was found to attenuate global SASP gene expression on oncogene-induced senescence (OIS), a persistent antiproliferative response that is associated with DNA damage and results from accumulation of driver mutations occurring on an oncogene or a tumor-suppressor gene [61]. Solid evidence has shown that melatonin prevents a sensor of DNA damage, poly(ADP-ribose) polymerase 1 (PARP-1), from interacting with a telomeric lncRNA, telomeric repeat-containing RNA (TERRA), during OIS. It is reported that TERRA is expressed from the telomeres on the ends of chromosomes and forms an integral part of telomeric heterochromatin together with the telomeric binding proteins to control genome integrity [62]. The observation that TERRA may facilitate the expression of SASP-associated genes on OIS suggests that melatonin may antagonize the TERRA–PARP-1-mediated induction of SASP by protecting telomeres.

Involvement of lncRNAs in Oncostatic Actions of Melatonin

Mounting evidence has demonstrated that lncRNAs drive many important cancer phenotypes [63]. Melatonin treatment was recently shown to induce the expression of the lncRNA CPS1 intronic transcript 1 (CPS1-IT1), which in turn attenuated hypoxia-inducible factor 1 alpha (HIF-1α) activity and resulted in suppression of the epithelial–mesenchymal transition (EMT) and liver cancer metastasis [64]. These results indicate that melatonin counteracts the progression of hepatic cancer via lncRNA CPS1-IT1-regulated suppression of EMT and implicated melatonin as a complementary treatment for combating malignancies.

Association of Melatonin-Regulated lncRNAs with Inflammasome Activation in Endothelial Pyroptosis

Differing from apoptosis and necrosis, pyroptosis is a proinflammatory programmed cell death where extensive proteolytic events occur through the formation of a multiprotein complex, the inflammasome, to mediate the processing and secretion of proinflammatory cytokines. In a recent study, the antipyrototic effects of melatonin on atherosclerotic endothelium were found to be mediated by a specific lncRNA, MEG3 [65]. MEG3 acted as an endogenous sponge by sequence complementarity to suppress the function of miR- 223 and to increase the expression of many inflammasome-related genes, ultimately inducing endothelial cell pyroptosis.

piRNA

piRNAs represent a large class of small ncRNAs named owing to their exclusive binding to PIWI proteins. They are highly abundant in the germline but also expressed in other tissues. Although less studied than miRNAs and siRNAs, the number of piRNAs is estimated to be much greater than that of miRNAs in most eukaryotic genomes [66]. Initially, the function of PIWI–piRNA pathways was proposed to silence the **transposons** in the germline. Recent findings have revealed their roles in epigenetic and post-transcriptional regulation of gene expression, which may govern germline specification, gametogenesis, stem cell maintenance, and genome integrity in various species [67–69].

A regulatory effect of a piRNA on MTNR1A expression has been documented [70]. In the study in question, a piRNA, piR_015520, was found to be expressed not only in the testes but also in the brain, for the first time; this shows that a human piRNA is transcribed in tissues different from the testes. The DNA sequence of piR_015520 is located in the first intron of the MTNR1A gene and forced expression of piR_015520 downregulated the expression of MTNR1A in HEK293 cells. Furthermore, piR_015520, unexpectedly, interacted with an uncharacterized RNA-binding protein but not with PIWI as assessed in an electrophoretic mobility shift assay. This line of evidence offers a new perspective on piRNA functioning as a regulator of melatonin signaling.

rRNA

In addition to crosstalk with regulatory ncRNAs, melatonin has been linked to modulation of ribosome biogenesis by downregulation of nascent pre-rRNA [71]. Ribosome biogenesis, involving the production of rRNAs and a great number of ribosomal proteins, is implicated in several fundamental aspects of cell biology, such as protein synthesis, cell growth, and proliferation [3]. Mammalian cells possess four types of cytoplasmic rRNA molecules (28S, 5.8S, 18S, and 5S). The 28S, 5.8S, and 18S rRNAs are derived from a single transcription unit (45S pre-rRNA) transcribed by RNA polymerase I and separated by two internal spacers [72]. Breast cancer cells with heightened tumor aggressiveness exhibit elevated generation of the 45S pre-rRNA, with activation of an alternative pathway of pre-rRNA production and augmented post-transcriptional methylation of particular regions located in the 28S rRNA [73]. Furthermore, many investigations have reported that attenuation of rRNA biosynthesis may serve as a target for anticancer therapies [74,75]. Of note, melatonin has been shown to suppress expression of the 45S pre-rRNA and a vital transcriptional regulator of rRNA genes, upstream binding factor (UBF), to blunt the viability of breast cancer cells [71]. These findings demonstrate an extra role for melatonin in tumor suppression through not just orchestrating the regulatory ncRNAs but also affecting the production of housekeeping ncRNAs (Figure 3).

Potential Directions for Future Research

Although the number of lncRNAs and piRNAs is found to outstrip the number of miRNAs, current understanding of the functional interaction between melatonin and ncRNAs is primarily focused on miRNAs, with much attention on silencing of downstream target genes. The importance of melatonin, if any, in the biogenesis and function of lncRNA and piRNA has remained largely elusive.

As an explosion of RNA-centric methods [76] occurred in recent years, a great diversity of lncRNA functionality has been documented. Among them, one of the most notable and prevailing is structure-encoded functions of lncRNAs through which secondary structures or modular functional domains are formed to coordinate versatile RNA–RNA, RNA–protein, and RNA–DNA interactions to regulate cellular events. In addition, there are some molecular features that distinguish certain lncRNAs from other classes of genes in terms of their biogenesis and turnover, although lncRNAs share similar expression programs with their

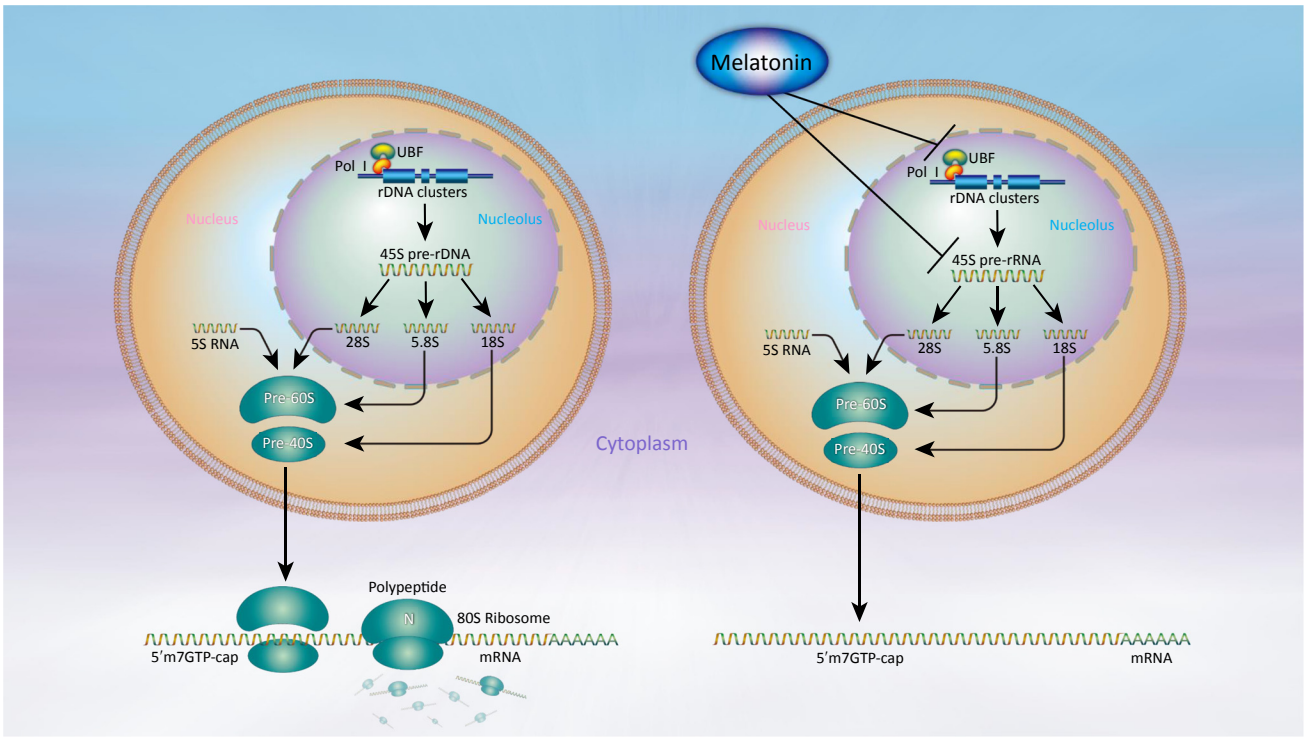

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Figure 3. Attenuation of Ribosome Biogenesis by Melatonin in Aggressive Breast Cancer Cells. Breast cancer cells sustain augmented protein synthesis and cell proliferation in part through extensive ribosome biogenesis. Production of ribosomes occurs in both the cytoplasm and the nucleolus and involves the assembly of four rRNA molecules (28S, 5.8S, 18S, and 5S) with a great number of ribosomal proteins (RPs). A single transcription unit (45S pre-rRNA) is transcribed in the nucleolus and then processed into 28S, 5.8S, and 18S rRNA, while 5S rRNA is transcribed in the nucleoplasm. Subsequently, 18S rRNA is assembled with small subunit RPs to generate the 40S ribosome precursor (Pre-40S), whereas 5.8S, 28S, and 5S rRNA, together with large subunit RPs, are united to form the 60S ribosome precursor (Pre-60S). Both the 40S and 60S precursors will be then transported to the cytoplasm where they become mature forms via the stepwise association and disassociation of many biogenesis factors (not shown here) and constitute the translation machinery with mRNAs and tRNAs. Under treatment with melatonin, the expression of 45S pre-rRNA and a key transcriptional regulator of rRNA genes, upstream binding factor (UBF), is attenuated, thereby restricting protein synthesis by and the proliferation of breast cancer cells.

protein-coding counterparts. For cellular localization, unlike mRNAs that reside specifically in the cytoplasm, lncRNAs can occupy the chromatin, subnuclear domains, the nucleoplasm, or the cytoplasm. In addition, differing from mRNAs that are mainly cleaved by 5′-to-3′ exonuclease degradation in the cytoplasm, turnover of lncRNA species is performed by the nuclear exosome [77] or cytosolic nonsense-mediated decay (NMD) [78]. These unique pathways and miscellaneous actions raise the complexity of lncRNA biology and prompt the interesting question of how many of them are possibly associated with melatonin signaling.

Another huge ncRNA class that remains poorly understood is piRNA. It has been shown that nascent piRNA transcripts are products of RNA polymerase II and potentially undergo 5′ capping, splicing, and 3′ polyadenylation [79]. However, it is unclear what the exact signals are (e.g., RNA modifications, modular motifs, secondary structures) that guide these transcripts from nuclear transcript production to piRNA processing in the cytoplasm. More importantly, several lines of evidence have indicated widespread roles of piRNAs outside the germline [67,69], but emerging functions of the PIWI–piRNA pathway beyond transposon silencing are largely unknown. A comprehensive examination using genome-wide **CRISPR–Cas9 screening** may provide detailed insights into nongonadal functions of piRNAs at different

developmental stages and in various tissues. These unsolved puzzles and their potential relationship to melatonin's actions will be an appealing objective to pursue in future research.

Concluding Remarks

The noncoding transcriptome and ncRNA interactome are now considered important regulators for most aspects of molecular and cellular biology. In this review we summarize the interplay between melatonin signaling and several types of regulatory ncRNAs, including miRNAs, lncRNAs, and piRNAs; this expands our knowledge of mechanisms underlying melatonin-regulated gene expression to a previously unforeseen extent. Also covered is an alternative role for melatonin in functional interactions with housekeeping ncRNAs, such as affecting the production of rRNAs in cancer suppression. These data provide clues for further clarification of ncRNA biology and additional mechanisms underlying the broad range of melatonin's actions (see Outstanding Questions).

Outstanding Questions

Does melatonin have a role in controlling lncRNA expression, as lncRNA promoters are in general as evolutionarily conserved as mRNA promoters in humans? What are melatonin-regulated lncRNA expression profiles in a specific cell/tissue type or developmental/pathological stage? How does melatonin orchestrate lncRNA transcriptomes?

Conversely, do lncRNAs modulate the fluctuation of melatonin signaling under various physiological and pathological conditions, in terms of the synthesis of melatonin and expression of melatonin receptors?

Do piRNAs serve as regulators of melatonin signaling, as their expression is not limited to the testis and their roles in epigenetic and post-transcriptional regulation of gene expression have been demonstrated?

Is melatonin functionally involved in ribosome biogenesis? What type of rRNA or protein is produced under the control of melatonin signaling?

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