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REVIEW

Melatonin modulates tumor metabolism and mitigates metastasis

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ABSTRACT

Introduction: Melatonin, originally isolated from the mammalian pineal gland, was subsequently identified in many animal cell types and in plants. While melatonin was discovered to inhibit cancer more than 5 decades ago, its anti-cancer potential has not been fully exploited despite its lack of serious toxicity over a very wide dose range, high safety margin, and its efficacy.

Areas covered: This review elucidates the potential mechanisms by which melatonin interferes with tumor growth and metastasis, including its ability to alter tumor cell metabolism, inhibit epithelial-mesenchymal transition, reverse cancer chemoresistance, function synergistically with conventional cancer-inhibiting drugs while limiting many of their side effects. In contrast to its function as a potent antioxidant in normal cells, it may induce oxidative stress in cancer cells, contributing to its oncostatic actions.

Expert opinion: Considering the large amount of experimental data supporting melatonin's multiple and varied inhibitory effects on numerous cancer types, coupled with the virtual lack of toxicity of this molecule, it has not been thoroughly tested as an anti-cancer agent in clinical trials. There seems to be significant resistance to such investigations, possibly because melatonin is inexpensive and non-patentable, and as a result there would be limited financial gain for its use.

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1. Introduction

Melatonin (N-acetyl-5-methoxy tryptamine) is functionally a highly diverse and multifaceted molecule. Originally identified and thought to be exclusive to the pineal gland, it is now apparent that this molecule is produced in many organs and possibly in the mitochondria of every cell. Melatonin is not unique to vertebrates but is also found in invertebrates and throughout the plant kingdom. It has been speculated that melatonin evolved 3.0 to 2.5 billion years ago in prokaryotes and that it has been retained throughout evolution [1]. Because of its circadian rhythm in the blood, with exclusively high nighttime levels in vertebrates, very early it was proposed to be involved in circadian and circannual variations in physiology, thereby functioning as both a clock and as a calendar [2,3]. While these functions remain important, melatonin has a plethora of other actions, perhaps impacting every cell in an organism. Because of its ability to beneficially modulate molecular physiology at the cellular level, melatonin has been referred to as a 'smart' molecule [4,5]. Melatonin's link to cancer was initially described over five decades ago. This field of research has matured rapidly, especially within the last 20 years. Many of the new findings related to melatonin-cancer interactions are summarized in this review.

2. Melatonin: antioxidant actions in normal cells

Oxygen-based free radicals are generated throughout a cell, more so in the mitochondria when electrons escape as they are transferred between the complexes of the electron transport chain. Melatonin functions in neutralizing highly reactive species in normal cells while, unexpectedly, if exaggerates oxidative stress in some cancer cells. These diametrically opposed actions afford it advantages as an anti-cancer agent.

Melatonin was discovered as a direct free radical scavenger 30 years ago [6]. In this study, melatonin's ability to function as a hydroxyl radical (·OH) scavenger was examined with the use of 5,5′-dimethylpyrroline N-oxide (DMPO) as the spin trapping agent. The $DMPO^{-}$·OH adduct formed was identified with the aid of an electrochemical detector attached to a high-performance liquid chromatography apparatus. The results were further verified by electron spin resonance spectroscopy (ESR) with a 1:2:2:1 ESR spectrum characteristic of the DMPO-·OH adduct being obtained; ESR is often considered as the most definitive means of identifying a molecule as a radical scavenger [7]. When melatonin was added simultaneously with DMPO to the reaction mixture, the ESR signal was inhibited in a dose-response manner, indicating that melatonin had quenched the ·OH. When two well-known radical scavengers, glutathione and mannitol, were tested in the same system, they proved less effective than melatonin. On the basis of

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Article highlights

- Melatonin is a highly versatile molecule since it functions as an antioxidant in normal cells and as a pro-oxidant in cancer cells.
- Melatonin's pro-oxidative action in cancer cells is one means by which melatonin kills cancer cells.
- Melatonin reverses Warburg-type metabolism in cancer cells and converts them to a more normal phenotype.
- Melatonin prevents the epithelial-mesenchymal transition of cancer cells thereby inhibiting the progression of cancer.
- Melatonin reverses chemo- and radio-resistance of cancer cells, making them more vulnerable to conventional drugs.

these and other findings, we concluded that melatonin is an important, endogenously-produced, non-enzymatic radical scavenger that likely also acts similarly within cells [6].

The results of this in vitro study were quickly supported by evidence that melatonin functions as a protection against oxidative stress *in vivo* [8–10]. Although both the *in vitro* and *in vivo* findings were initially met with considerable skepticism, the results were soon verified in other laboratories [11,12]. Currently, melatonin is well-known as a significant radical scavenger in normal cells, especially under conditions of elevated oxidative stress [13,14].

Of equal importance, however, is that the by-products that are formed when melatonin neutralizes a partially reduced oxygen derivative (a radical, also collectively referred to as reactive oxygen species or ROS), are likewise radical scavengers (Figure 1). The first of these to be identified was cyclic-3-hydroxymelatonin. Like the parent molecule, it is effective as a ROS scavenger [15]. Other metabolites that are also direct radical scavengers and inhibitors of oxidative stress include N-acetyl-N-formyl-5-methoxykynuramine (AFMK) and N-acetyl-5-methoxykynuramine (AMK) [16,17]. Some of these metabolites are even better scavengers than melatonin [18–20]. The differential actions of melatonin's metabolites as ROS scavengers make it impossible to attribute a specific percentage of the reduced oxidative stress to melatonin *per se*. The sequential neutralization of radicals by melatonin and its metabolic kin is referred to as the antioxidant cascade (Figure 1) [21]. Via this pathway, melatonin incapacitates multiple radical products in contrast to other well-established antioxidants that typically neutralize a single free radical. Besides protecting healthy macromolecules from destructive ROS, at least one molecule, i.e. DNA, when oxidatively damaged, can be repaired by enzymes stimulated by melatonin [22].

Often overlooked as a molecule with antioxidant activity is the chemical intermediate formed during melatonin biosynthesis from serotonin, N-acetylserotonin (NAS). In recent years, NAS has been espoused to be neuroprotective with both antioxidant and anti-inflammatory activity [23]; NAS may be superior to melatonin in countering free radical damage [24]. The evidence for NAS functioning as a radical scavenger is indirect and, overall, the amount of data supporting its antioxidant activity is not as compelling as for melatonin and its derivatives. Nevertheless, NAS should not be ignored as to its benefits in warding off oxidative damage in light of evidence that melatonin can be reverse metabolized to NAS [25]; this backward conversion is a consequence of the activity of CYP1B1 (cytochrome P450 family 1 subfamily B member 1) and CYP1A2 (cytochrome 450 family 1 subfamily A member 2) [26], possibly driven by the aryl hydrocarbon receptor (AhR) (Figure 1). CYP1B1 is a monooxygenase involved in ROS generation and induction of ferroptosis [27]; thus, an increase in the activity of this enzyme and consequent redox imbalance in cancer cells could inhibit their survival. Anderson [28,29] presents a strong case for the NAS:melatonin ratio being critical for the optimal functioning of mitochondria, an organelle in which both NAS and melatonin are formed. An NAS:melatonin ratio that favors the former changes the intramitochondrial microenvironment, leading to perturbations in sirtuins, the AMPK-mTOR pathway and oxidative stress/PARP interactions, the combined functions of which could alter mitochondrial oxidative homeostasis. NAS is also a brain-derived neurotrophic factor (BDNF) mimic, as indicated by its activation of the BDNF receptor, tyrosine receptor kinase B (TrkB). However, TrkB activation promotes the proliferation and survival of cancer stem-like cells, indicating that the release of NAS from any tumor microenvironment cell may enhance tumor survival [30].

Antioxidants can function in the reduction of oxidative stress by means other than scavenging ROS. In many cases, oxidative damage is prevented when enzymes metabolize toxic oxygen derivatives to less toxic or innocuous products. Thus, an antioxidant often functions in the protection of macromolecules from ROS by stimulating antioxidative enzymes; important enzymes in this category include glutathione peroxidase (GPx), glutathione reductase (GPx), catalase (CAT), both cytosolic and mitochondrial superoxide dismutase (SOD), peroxiredoxin (PRDx) (Figure 2) and glutamate-cysteine ligase (GCL), which enhances glutathione levels [31–35]. Melatonin also inhibits nitric oxide synthase (NOS), a pro-oxidative enzyme [36], and to deactivate hypochlorous, a free radical generator [37]. These indirect actions of melatonin in terms of reducing oxidative stress accumulated within a decade after melatonin was identified as a radical scavenger. Melatonin's ability to modulate the activity of enzymes that reduce the degree of oxidative stress is believed to be receptor-mediated in contrast to the direct scavenging functions which are receptor-independent, although these statements require further experimental verification.

Because of their high reactivity, ROS travel infinitesimally short distances before they interact with another molecule and their half-lives are equally extremely brief, often in the microsecond (10^{-6} s) time scale when measured in pure chemical systems [38]. The interaction of a radical scavenger with a powerful oxidizing agent also depends to a degree on the molecular composition of the microenvironment where the reaction takes place [39]. Also, molecules can be compartmentalized, e.g. by means of biomolecular condensates, which determine the rate at which nearby molecules may interact [40]. Nevertheless, it is generally agreed upon that a radical scavenger must be in the proximate vicinity of an electron-deficient reactant for it to be neutralized before it inflicts

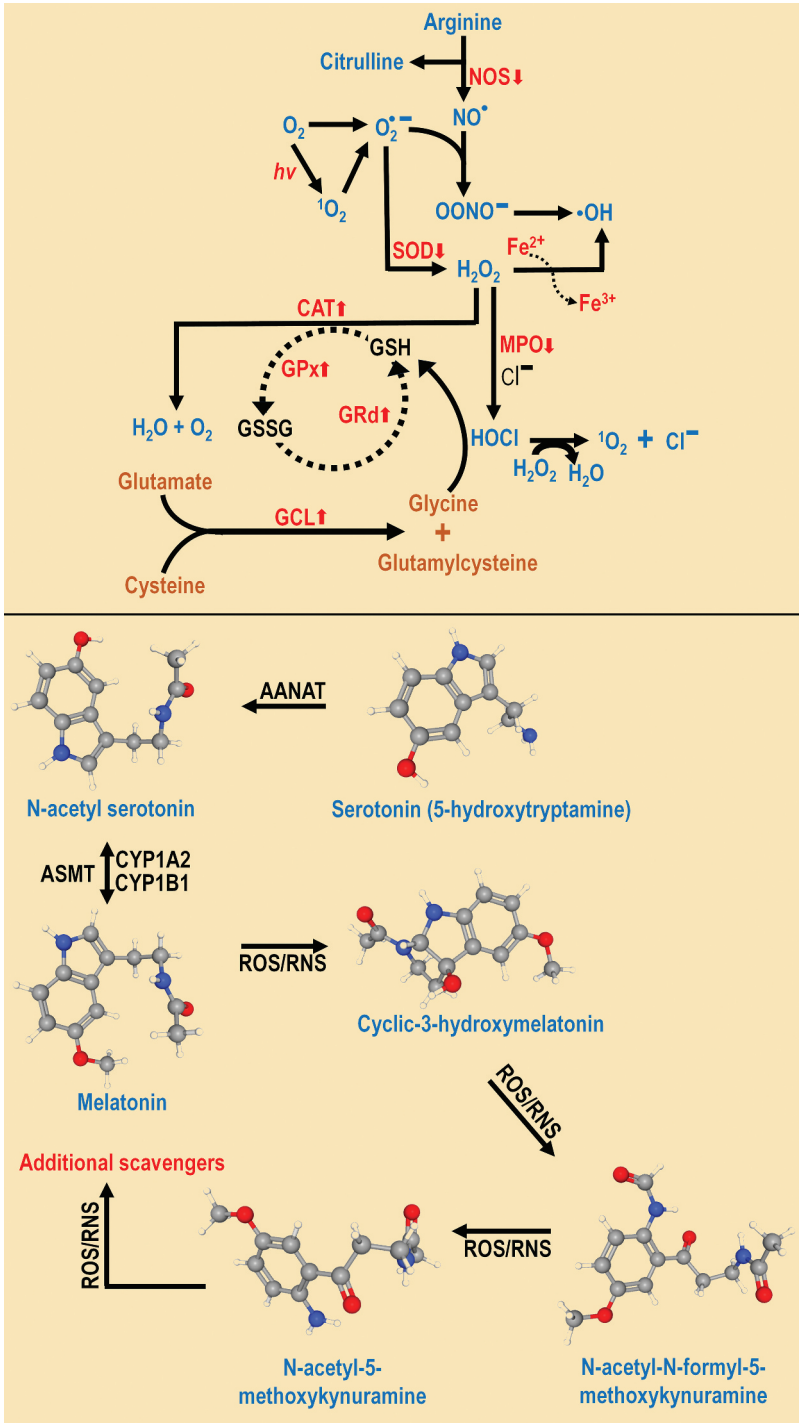

Figure 1. Free radicals, melatonin and melatonin metabolites as radical scavengers. This two-part figure illustrates (Top) the reactive oxygen species (ROS) and reactive nitrogen species (RNS) (some are also known as free radicals) that indiscriminately damage essential molecules that lead to nitro/oxidative stress. Although they can be generated at multiple sites within cells, ROS are often abundantly produced in the mitochondria as a result of OXPHOS and can contribute to cancer initiation, growth and metastasis. It is the function of antioxidants to directly scavenge the ROS or enzymatically metabolize them to innocuous species. Melatonin, along with its metabolites including cyclic-3-hydroxymelatonin, N-acetyl-N-formyl-5-methoxytryptamine and N-acetyl-5-methoxytryptamine, are all radical scavengers (Bottom); this series of metabolites is known as the antioxidant cascade which is believed to be functional in normal cells. The precursor of melatonin, N-acetylserotonin, is also a documented antioxidant in normal cells. In cancer cells, reduced levels of these antioxidants contribute to oxidative stress. In the top illustration, up arrows indicate stimulation and down arrows indicate inhibition. AANAT, aralkyl amine-N-acetyltransferase; ASMT, acetylserotonin-O-methyltransferase; CAT, catalase; CYP1A2, cytochrome P450 family 1 subfamily A2 member; CYP1B1, cytochrome P450 family 1 subfamily B1 member; GCL, glutamine cysteine ligase; GPx, glutathione synthetase; GRd, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; hv, high energy visible light; MPO, myeloperoxidase; NOS, nitric oxide synthetase; SOD, superoxide dismutase.

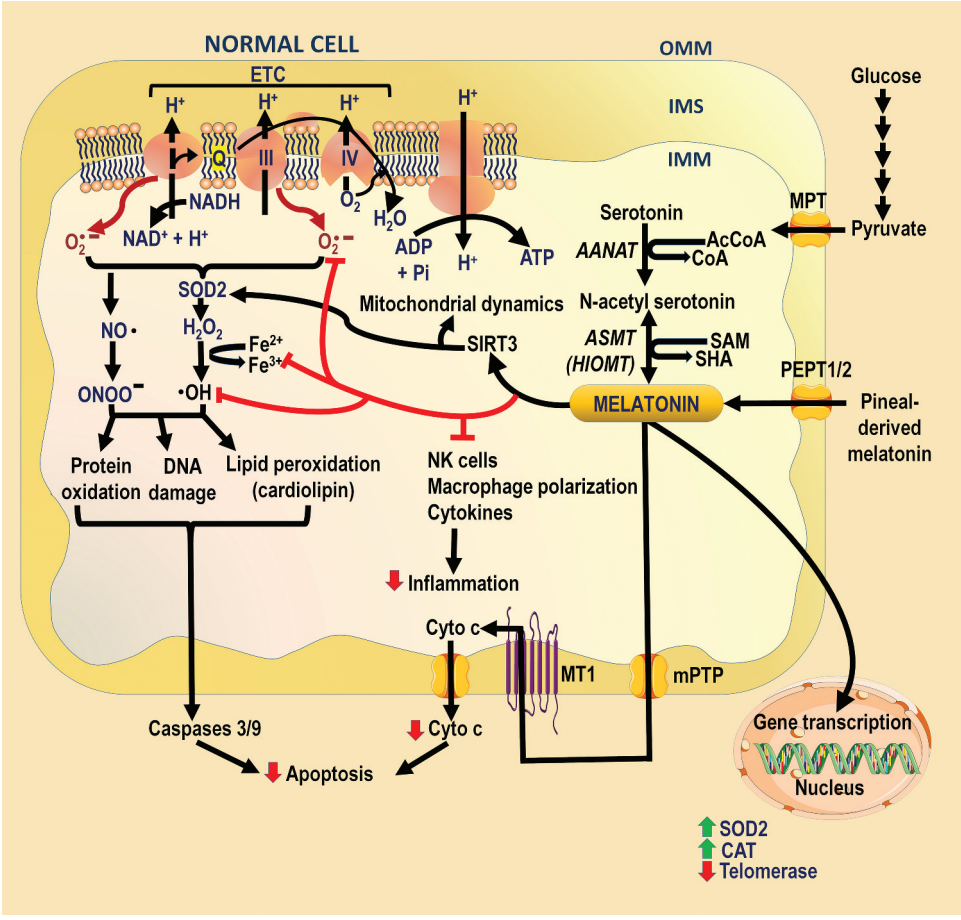

Figure 2. An overview of the synthesis of melatonin in a mitochondrion and its intramitochondrial actions in a normal (non-pathological) cell. the pathway for the synthesis of melatonin from serotonin in mitochondria is the same as in the pineal gland; serotonin is first N-acetylated by the enzyme aralkylamine-N-acetyltransferase (AANAT) to N-acetylserotonin which is then O-methylated to melatonin. In addition to mitochondrial synthesis, melatonin can also be taken up from the systemic circulation via the PEPT1/2 receptor. AANAT, which is rate limiting in melatonin production, requires the co-substrate, acetyl coenzyme (AcCoa), along with serotonin for the synthesis of N-acetylserotonin. AcCoA is a product of pyruvate, the terminal metabolite of glucose metabolism in the cytosol. In cancer cells where Warburg-like metabolism occurs, pyruvate does not enter the mitochondria so it does not serve as a source of AcCoA. As a result, melatonin production is negatively impacted and in cancer cells; melatonin levels in cancer cells are reported to be only half those in normal cells. Many of the actions of melatonin within mitochondria relate to its ability to influence redox homeostasis. In normal cells, as depicted here, melatonin reduces apoptosis. In cancer cells, often opposite actions of melatonin are apparent (see text for details). AcCoA, acetyl coenzyme A; ADP, adenosine diphosphate; ASMT, acetylserotonin-O-methyltransferase (HIOMT – hydroxyindole-O-methyltransferase – the former name of ASMT); ATP, adenosine triphosphate; CAT, catalase; CoA, coenzyme A; Cyto c, cytochrome c; ETC, electron transport chain; IMM, inner mitochondrial membrane; IMS, intermembrane space; NAD+, nicotinamide adenine dinucleotide oxidized; NADH, nicotinamide adenine dinucleotide reduced; NK cells, natural killer cells; mPTP, mitochondrial membrane potential; MT1, melatonin receptor 1; OMM, outer mitochondrial membrane; PEPT ½, oligopeptide transporter; Pi, phosphate; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; SIRT3, sirtuin 3; SOD2, superoxide dismutase 2 (mitochondrial superoxide dismutase).

damage. Thus, since melatonin is present in mitochondria, major ROS-producing organelles, it is ideally situated to provide on-site antioxidant protection.

Free radicals are either the result essential metabolic events that normally occur in cells, e.g. electron transport chain (ETC), activity of oxidases, etc., or they are a consequence of external pathological influences. The ETC continually fuels ROS generation when it mishandles electrons as they pass through the complexes of the ETC in the inner mitochondrial membrane, in particular at complex I (NADH dehydrogenase) and complex III (ubiquinone cytochrome c reductase) (Figure 2). Thus, mitochondria are the likely site of production of a significant portion of damaging radicals. Therefore, the presence of high concentrations of a radical scavenger in these organelles would seem to be a good strategy to reduce the associated damage and maintain mitochondrial wellbeing [41]. Although measurements of the level of melatonin in mitochondria are limited, available data indicate that, among subcellular organelles, the highest concentration of melatonin is in the mitochondria [42]. This may be a result of its intramitochondrial synthesis [43] or the ease with which mitochondria sequester melatonin from blood [44], or more likely to both. Importantly, cancer cells damaged by oxygen-based reactants have only about half the level of melatonin relative to that in normal cells [45,46]; thus, protection of this critical organelle in cancer cells against oxidative stress is presumably likewise reduced.

Having optimal levels of melatonin in mitochondria of non-pathological cells to resist their continuous bombardment by free radicals originating from the ETC would

seem essential in limiting what has come to be known as mitochondrial diseases. Conversely, reduced melatonin concentrations in mitochondria of cancer cells may be a good thing in that it normally contributes to the cancer cell-killing potential of melatonin (see below).

3. Melatonin: pro-oxidant actions in cancer cells

The vast majority of studies unequivocally confirm the antioxidant and oxidative stress-lowering capacities of melatonin resulting from its function as a direct free radical scavenger, its modulation of antioxidative and pro-oxidative enzymes, and its ability to chelate transition metals which generate, via the Haber-Weiss or Fenton reactions, the highly toxic ·OH (hydroxyl radical) [18]. However, contrary claims have also been made, the first of which was reported by Medina-Navarro and colleagues [47]. They found that melatonin exhibited pro-oxidant activity in a cell-free system where it increased damage to proteins and lipids. In systems containing either cultured Jurkat cells [48] or human leukemia cell lines [49], both of which have a pathological phenotype, melatonin, at select concentrations, induced free radical generation and cytotoxicity. The outcomes of the early studies, all of which were performed in vitro, generally indicated melatonin promoted ROS production in cancer cells, and initiated cell death pathways; these changes were usually associated with pharmacological levels of melatonin in the incubation media [20]. The conventional membrane receptors, MT1 and MT2, seem not to have been involved in stimulating free radical generation since, luzindole, a classic melatonin membrane receptor blocker, did not change the outcome of the pro-oxidant studies [50].

The initial attempt to explain what the authors referred to as the antioxidant and conditional pro-oxidant actions of melatonin was provided by Zhang and Zhang [20]. They summarized the experimental situations, none of which were carried out *in vivo*, in which melatonin seemingly promoted free radical generation. As the authors point out in their summary of these early studies, the results were highly variable, and outcomes may have been related to the concentration of melatonin used or to the duration of treatment. There was certainly no uniform treatment during which the pro-oxidant actions were observed.

Some data were presented [20], however, to explain how melatonin could change its function from being highly antioxidant to one that generated ROS. When melatonin-treated U937 cells, a human myeloid leukemia cell line, were also exposed to chlorpromazine, a specific inhibitor of calmodulin, a rise in ROS generation was apparent [50]. Calmodulin, a multifunctional agent, binds melatonin with low affinity [51]. As envisioned, calcium-dependent PLA2 is normally bound in its inactive form by calmodulin. High concentrations of melatonin competitively frees PLA2 from calmodulin, stimulating arachidonic acid (AA) release, which, in turn, promotes 5-lipoxygenase (5-LOX) activity, resulting in excess ROS formation. Similarly, iPLA2 (calcium-independent phospholipase 2) or pharmacological suppression of 5-LOX activity also potentially enhances melatonin's pro-oxidant action in cancer cells. While ample evidence shows that melatonin alters calmodulin in various ways [18–20,52], there are no convincing follow-up studies supporting the hypothesis that melatonin drives ROS generation in pathological cells resulting in their death.

An alternative explanation for elevated cellular free radicals after melatonin treatment was advanced [53]. The authors found that melatonin transiently increased the transcription and translation of neuronal nitric oxide synthase (nNOS) which caused a reduction in the efficiency of oxidative phosphorylation (OXPHOS), a lowering of the mitochondrial membrane potential, and a shift in cellular metabolism to glycolysis. They surmised that electron leakage was enhanced due to the inefficient OXPHOS, leading to the elevated chemical reduction of adjacent oxygen molecules. Although the authors did not know it at the time, the metabolic shift from OXPHOS to glycolysis likely reduced the intramitochondrial concentrations of melatonin [54–56], allowing free rein for the generated ROS to damage and kill the cancer cells. If this theory is valid, free radical generation may not only be exaggerated in cancer cells, but because of the reduced cellular melatonin values as have now been reported [45,46], the produced ROS are uncontested and are available to damage essential molecules.

Both cytostatic and cytotoxic effects of melatonin have been observed when tested against various cancers; the basis for these differential effects remains unknown. Melatonin, in contrast to its actions in normal cells, can be pro-oxidant and pro-apoptotic in many cancer cells. However, with regard to cancer cell death, there is significant variability in how tumor cells respond to melatonin; the specific response may depend on the concentration of melatonin or on some aspect of the microenvironment in which the cells are situated. Melatonin-mediated free radical generation in tumor cells could cause apoptosis by impacting either the intrinsic or extrinsic pathway of programmed cell death (Figure 3) [57]. Elevated ROS likely induces apoptosis via the mitochondrial (intrinsic) pathway. This occurs when free radicals modify the function of the mitochondrial permeability transition complex, leading to the translocation of Bax and Bak, proteins that promote the release of cytochrome c from the mitochondria into the cytosol and thereby initiate a cascade of caspase-mediated cell death [58]. Extrinsic cell death pathway occurs when external ligands such as Fas/CD95 or TNF-α (tumor necrosis factor α) activate cell surface receptors which stimulate the development of the death signaling complex (DISC) and eventual stimulation of caspases-3 and −7, resulting in cancer cell suicide [59]. These findings suggest that depressing antioxidant enzyme levels in cancer cells, i.e. allowing free radicals to plunder at will, would aid in killing the tumor. Recent studies report that melatonin does in fact repress SOD, CAT, GPx, GRd, and glutathione S-transferase in colon cancer cells, resulting in an enhancement of oxidatively-damaged molecules and their death [60,61]. Oxidative stress is an important inhibitor of PTEN-induced kinase (PINK)1/mitophagy coupled to the upregulation of major histocompatibility complex (MHC)-1, which enhances CD8+ T cell-driven apoptosis in cancers and other 'immune-mediated' conditions, highlighting the intimate link between the oxidative state in

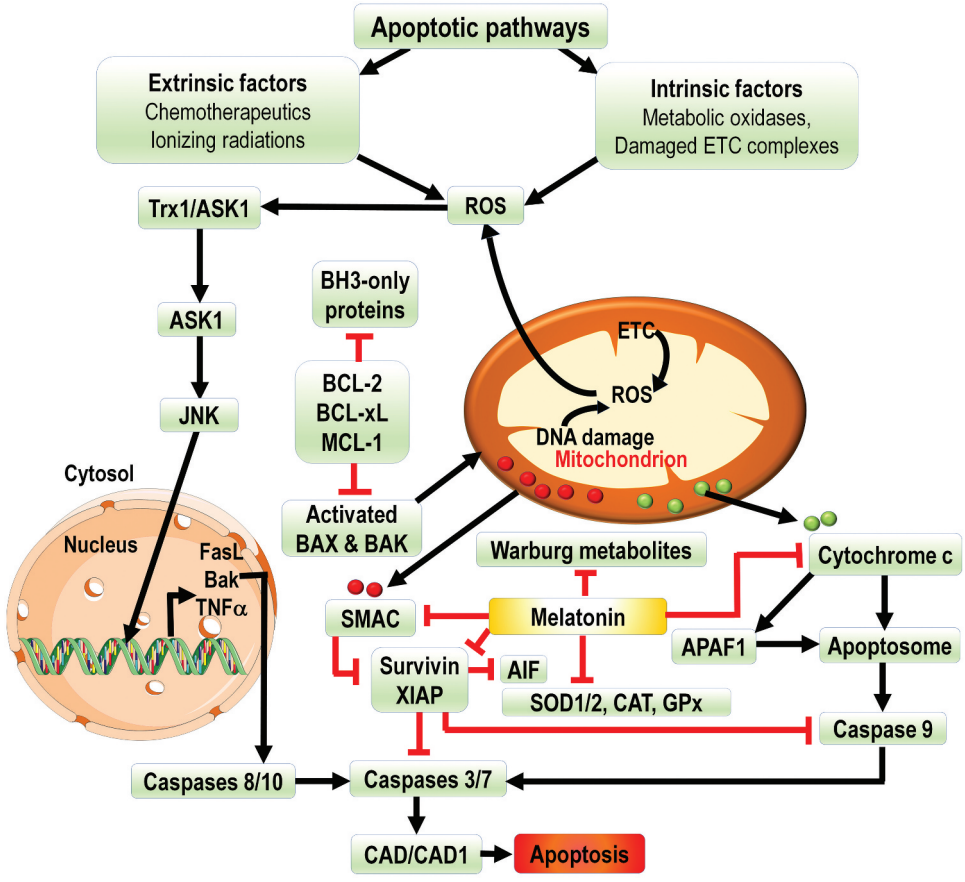

Figure 3. Presumed mechanisms involved in melatonin-mediated induction of apoptosis in cancer cells. Normally, cancer cells utilized a number of ploys to escape undergoing apoptosis such as upregulating survivin and XIAP (X-linked inhibitor of apoptosis protein). These, in turn, inhibit the caspases that mediated apoptosis. Both of these potent survival factors are repressed my melatonin in cancer cells. Moreover, melatonin inhibits both SMAC (second mitochondria-derived activator of caspase) and cytochrome c which, via downstream processes, also induce the execution caspases that lead to apoptosis. The upregulation of TNFα (tumor necrosis factor α) contributes to the extrinsic pathway of apoptosis; TNFα along with other proinflammatory agents is inhibited by melatonin in many situations. The net result of these actions and others is a reduction in the death of cancer cells, allowing the tumor to continually enlarge. AIF, apoptosis inducing factor; APAF1, apoptotic protein activating factor 1; Ask-12, apoptosis signal-regulating kinase 1; Bak, nuclear encoded proapoptotic factor; BCL-2, B-cell lymphoma 2-gene; BH3-proteins, proapoptotic family members; CAD, caspase activated DNases; CAT, catalase; ETC, electron transport chain; FasL, Fas ligand; GPx, glutathione peroxidase; JNK, jun N-terminal kinase; MCL-1, myeloid cell leukemia 1 protein; ROS, reactive oxygen species; SOD ½. superoxide dismutase 1 and 2; Trx1, thioredoxin 1.

tumors and intercellular interactions in the tumor microenvironment [62]. In addition to passively enhancing oxidative stress in colorectal cancer, melatonin decreased the mRNA and protein levels of two key inhibitors of apoptosis, XIAP (X-linked apoptosis protein) and survivin, thus further increasing the likelihood of the cancer cells to undergo programmed cell death (Figure 3).

Sirtuin3 (SIRT3), a class III histone deacetylase, which is located primarily in the mitochondrial matrix, has major roles in influencing intracellular metabolism and in regulating mitochondrial oxidative stress. The latter is achieved when melatonin upregulates the SOD2 transcription factor, FOXO3a, resulting in the increased dismutation of the $O_2^{\cdot-}$ to H_2O_2 [63]. In HeLa cancer cells, melatonin exaggerates oxidative stress generated by the cytotoxin, shikonin (SHK), leading to apoptosis [64]. The effects of the combination of SHK and melatonin are reversed by treatment with conventional antioxidants, indicating the involvement of ROS. These findings are consistent with the pro-oxidant actions of melatonin during the process of killing cancer cells. Somewhat in contrast to these results, we recently reported that in the Lewis mouse lung cancer model (using three different cell lines), melatonin treatment stimulated SIRT3, increased the activity of pyruvate dehydrogenase complex, and reprogrammed cell metabolism by converting the metabolic profile from cytosolic glycolysis (Warburg-type metabolism) to mitochondrial OXPHOS [65]. This change not only elevated the activities of complexes I and IV as well as (adenosine 5′-triphosphate; ATP) synthesis but also enhanced intramitochondrial oxidizing environment, resulting in cancer cell damage and death. Switching the metabolic profile of pathological cells is a common feature of melatonin [54,66]. While the processes by which melatonin kills cancer cells differed between studies [64,65], the end-result, i.e. cancer cell death due to elevated oxidative stress, was the same.

Sirtuin 1 (SIRT1) is also linked to melatonin's ability to suppress tumor proliferation and growth. In non-pathological cells, melatonin upregulates SIRT1 activity [67]. In contrast, treatment of osteosarcoma cells with melatonin diminished SIRT1 activity, thereby increasing the pro-oxidant profile and its cancer-killing action. Pharmacological manipulations of SIRT1, i.e. inhibition or stimulation, supported the notion of

melatonin killing cancer cells by exaggerating free radical generation [68].

Akt, a serine/threonine kinase, has wide-spread actions in normal cells including the inactivation of pro-apoptotic proteins, maintenance of oxidative homeostasis, regulation of glucose metabolism, and phosphorylation of a range of proteins such as glycogen synthase kinase-3β (GSK-3β) [69]. Melatonin alters Akt activity in both non-cancer and cancer cells. In SK-MEL-1 human melanoma cells, melatonin enhanced GSK-3β dephosphorylation by inhibiting Akt which resulted in a marked increase in ROS generation [70]. Moreover, Nrf2 (nuclear erythroid 2-related factor 2) was downregulated; this contributed to the oxidizing environment of the cells by diminishing antioxidant enzyme responses. Thus, as in several other experimental models, melatonin's negative actions on cancer cells stem, at least in part, from its stimulation of pro-oxidant actions, with a concurrent diminished antioxidant state.

4. Melatonin changes cancer cell metabolism

In multicellular organisms, the majority of cells when they reach their differentiated state, generate ATP required for proliferation and other cellular processes via OXPHOS. In contrast, many cancer cells adopt a seemingly inefficient but rapid alternate means of producing ATP. When this occurs, energy is primarily generated during cytosolic glycolysis; it is not always clear how glycolysis for ATP synthesis benefits cancer cells but they often flourish using this pathway. This metabolic adjustment made by cancer cells was discovered a century ago [71], and is conventionally referred to as the Warburg effect or aerobic glycolysis as it occurs even when there is ample oxygen to support mitochondrial OXPHOS. During this process, pyruvate, the end product of glucose metabolism does not enter the mitochondria but rather undergoes fermentation to lactate in the cytosol and is subsequently released from the cells, acidifying the tumor microenvironment; this affords the cancer cells advantages in terms of invasion and metastasis [72]. Warburg-type metabolism is reversible and plays important roles in epithelial-mesenchymal transition (EMT) of cancer cells [73].

In 2014, Blask and coworkers [74], while investigating the metabolism of human breast cancer xenografts growing in immune-compromised nude rats, collected tumor tissues at 4-hour intervals over a 24-hour light:dark cycle. They observed pronounced 24-hour rhythms of all tumor metabolic parameters measured such as glucose uptake or lactate release, (Figure 4) indicating major differences between tumor metabolism during the day and at night. Their findings suggested that cancers were metabolically highly active during the day when they employed Warburg-type metabolism but were metabolically quiescent at night. The authors also measured blood-melatonin rhythm in tumor-bearing rats and, as expected, melatonin levels were highly elevated at night relative to those measured during the day. Suspecting that the melatonin rhythm was driving the marked variation in tumor cell metabolism, the nocturnal melatonin rise was suppressed in another group of rats by exposing them to light at night. Profound changes in tumor metabolism occurred such that the cancer cells maintained very high activity throughout the 24-hour period; the tumors also grew at a faster rate compared to those in animals maintained in a conventional light: dark cycle (Figure 4).

The marked circadian rhythms in Warburg-type metabolic parameters showed that tumors exhibited different metabolic profiles during the day than at night, and that the differences temporally correlated with circulating melatonin levels. Thus, melatonin switched the tumors from using Warburg metabolism during the day to typical OXPHOS at night. What this means is that some tumors may be only cancerous part-time, and function normally at other times [75]. These results have important implications for cancer research wherein most investigations are performed on tumors collected during the day, perhaps providing misleading information. Also, when cancer metabolism is investigated using cultured cells, which are not exposed to the circadian melatonin rhythm, critical data will likely be lost. Finally, these data lend credence to the observations that nocturnal light exposure increases cancer frequency in humans due to suppression of melatonin [76].

The reversal of aerobic glycolysis in cancer cells by melatonin has been confirmed in several cancer cell types. Unlike in the original publication [74], which depended on the rise of endogenous melatonin to switch Warburg-type metabolism to OXPHOS, subsequent studies used exogenously administered melatonin and achieved the same result. For example, orally administered melatonin overcame aerobic glycolysis in leiomyosarcoma xenografts [77,78]. Concurrently, every metabolic parameter of the tumors reflected the switch to OXPHOS and the previously identified cancer cells became non-pathological. Changes associated with the metabolic shift induced by melatonin included reduced uptake of linoleic acid (a growth factor for cancer cells) and loss of its conversion to the mitosis-mediating agent, 13-hydroxyoctadecadienoic acid (13-HODE), along with suppressed DNA synthesis and decreased phospho-activation of several key enzymes (Akt, GSK3β and ERK ½). Simultaneous administration of S20928, a membrane melatonin receptor blocker, reversed the actions of melatonin on cancer cell metabolism.

Dauchy and coworkers [79] employed a different approach to determine melatonin association with aerobic glycolysis in hepatoma 7288CTC xenografts. To test whether the quality of daytime light would change the pattern of the nocturnal melatonin rise, they exposed tumor-bearing rats to blue-enriched light (465–485 nm) during the day and to conventional darkness at night. What must have been unanticipated findings, they observed that the nocturnal melatonin peak was 7-fold higher because of blue daytime light exposure. They measured many of the same parameters as estimated by Mao et al [78] in their previous publication. While melatonin reprogrammed the metabolic activity of the hepatoma cells to a more normal phenotype, the more robust rise in nighttime melatonin levels was not more effective in reducing aerobic glycolysis than the regular nighttime increase.

Collectively, these findings have important clinical implications. We described tumors in cancer patients that are less cancer-like at night and more cancer-like during the day as part-time cancers [75]. As envisioned, during

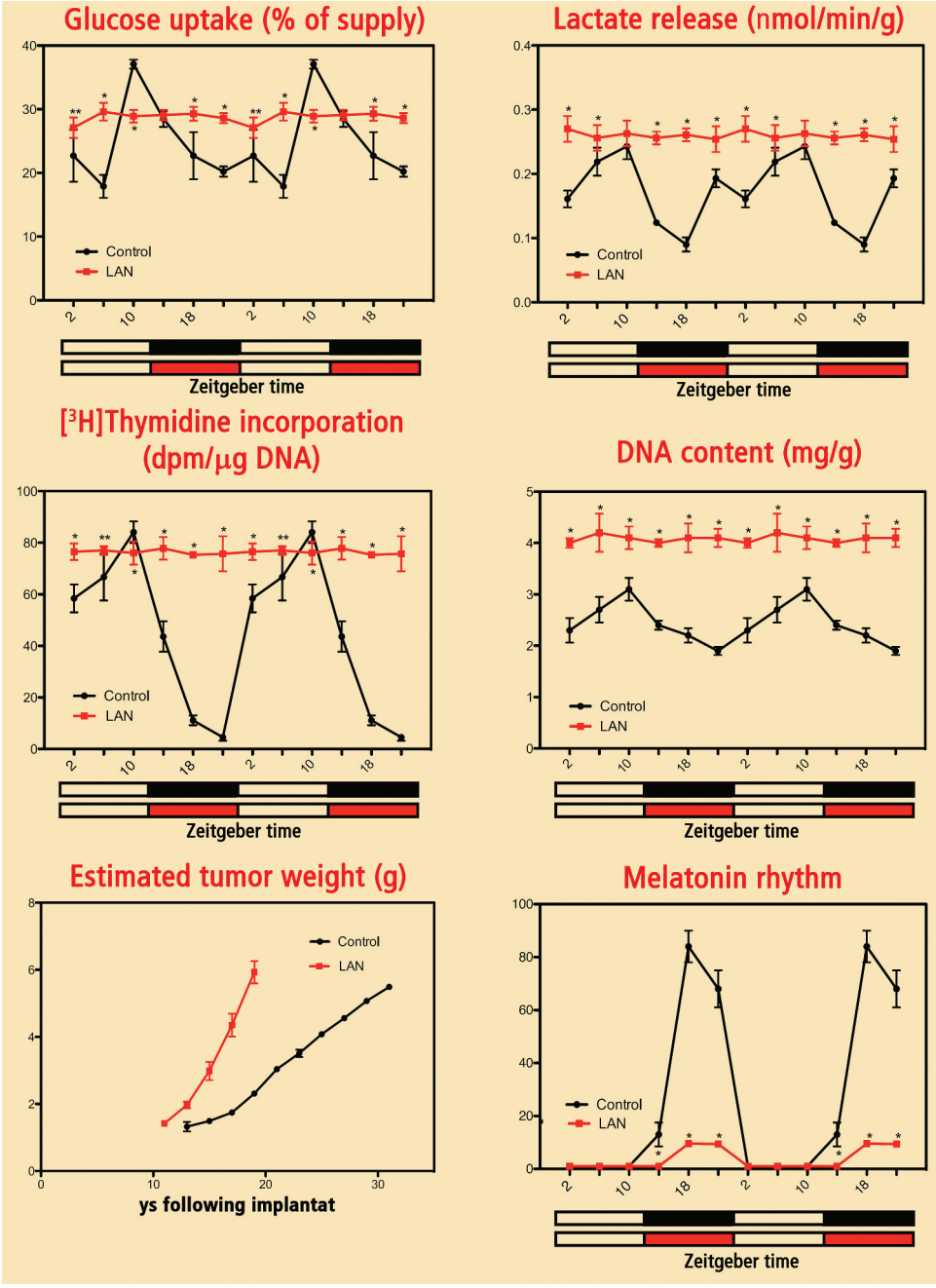

Figure 4. Circadian variations (black line) in metabolic parameters of human MCF-7 breast cancer xenografts growing subcutaneously in nude rats. The tumors were collected at 4-hour intervals over a light:dark cycle (alternating light-dark bar below the figures) for the measurements that were made. Besides the data shown in this figure, many other measurements were made and all varied similarly. Clearly, the daily light period is associated with high cancer cell metabolism while during darkness the tumors are relatively metabolic dormant. The high glucose uptake and elevated lactate release during the day indicate that during the light period, the tumors were undergoing Warburg-type metabolism while at night they apparently had switched to OXPHOS. Thus, they seem to be functioning with a cancer phenotype during the day; at night the cells had a more normal metabolic state. These rhythms were obviously being driven by the endogenous melatonin rhythm since suppressing circulating nocturnal melatonin levels with light at night (LAN) (the alternating white and red bar under the figures) led to a persistent high metabolic activity (red line) throughout the 24-hour period; moreover, these tumors grew more rapidly (bottom left). The weight of the tumors was estimated by measuring the tumors and performing the appropriate calculations. Data reconfigured from Blask et al. [74] and printed with permission (originally published under the Creative Commons Attribution license).

the day at least select cancers exhibit aerobic glycolysis and are maximally proliferative thus exhibiting more rapid growth; they undergo a melatonin-mediated switch to OXPHOS at night and to a more normal cell phenotype, growing more slowly. Thus, if melatonin is used as a treatment for cancer, it likely should be given during the day to suppress tumor growth rather than at night when it is usually taken to promote sleep. Also, hospitalized cancer patients (and others as well) should be maintained daily in the dark as long as convenient to ensure a robust nocturnal melatonin rise; this could be readily achieved with eye shades. This only applies to individuals at an age

where they normally exhibit a nocturnal melatonin increase. Most individuals lose the ability to produce melatonin in advanced age, a change that also gives cancer cells an advantage in terms of growth and metastasis.

Warburg-type metabolism is often identified as a hallmark of cancer cells [80]. The studies summarized here show that this characteristic metabolic type may, however, be present only about half the time in tumor cells. Thus, placing Warburg metabolism on a list of identifying features of tumor cells, without its qualification to daytime or nighttime measurements, may not be valid. Metabolic reprogramming to aerobic glycolysis is an adaptation that allows cancer cells to survive and proliferate at a rapid pace, maintain their tumorgenicity, resist attacks from immune cells, retain their cell stemness, become resistant to chemotherapies, and maintain the cellular microenvironment in a state that facilitates cell invasion and metastasis. Additionally, epithelial-mesenchymal transition, a requirement for the transition of normal cells to the cancer phenotype, is also dependent on nutrients provided by aerobic glycolysis [73]. Even though the specific mechanisms by which melatonin governs the type of glucose metabolism utilized and limits cancer development is unknown, the fact that an endogenously-produced molecule can convert cancer cells to a less cancerous state by directly or indirectly modulating each of these features should find great interest among experimental and clinical oncologists.

5. Melatonin inhibits the epithelial-mesenchymal transition

Epithelial-mesenchymal transition is a reversible process characteristic of most carcinomas. This change imparts important advantages to the tumor cells including making them mesenchymal-like (stem cell phenotype). The presence of stem cells in tumors supports tumorigenesis and enhances

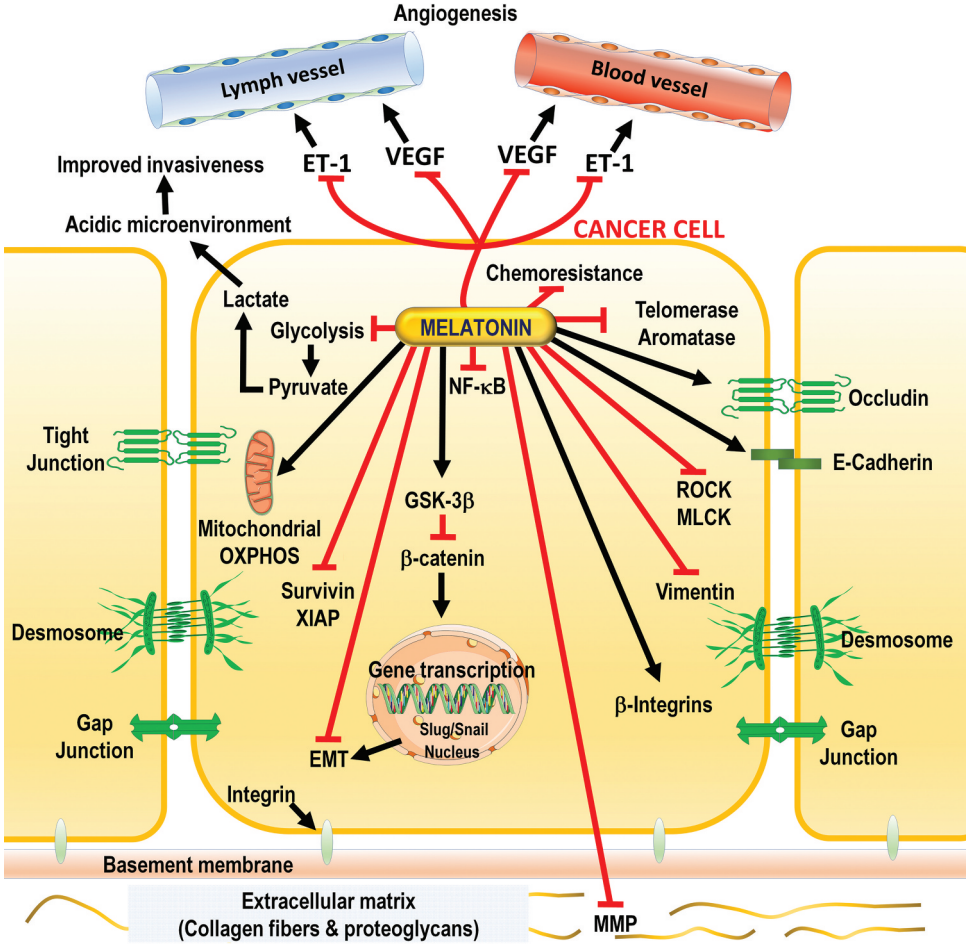

Figure 5. A summary of the proposed actions by which supplemental melatonin limits cancer cell metastasis. Melatonin, when taken orally or given via any other route, enters the blood and is absorbed by both normal and cancerous cells. Experimental evidence is compelling that melatonin functions via multiple means to impede cancer metastasis while simultaneously preserving the well-being of normal cells by protecting them from oxidative stress. Some of the actions utilized by melatonin to reduce the likelihood of cancer cells extravasating into the vascular system and establishing growth sites in remote organs include, a) preventing the degradation of cellular adhesion molecules (occludin, E-cadherin, integrin, etc.) that anchor cells to each other and to the basement membrane, b), reducing cytoskeletal re-organization, which improves the ability of cancer cells to invade adjacent tissues, by downregulating ROCK (Rho-associated kinase), MLCK (myosin light chain kinase) and vimentin, c), upregulating GSK-3β (glycogen synthesis kinase β) to inhibit β-catenin to prevent the epithelial-mesenchymal transition (EMT) by inhibiting slug and snail, d), preventing the breakdown of the extracellular matrix by suppressing matrix metalloproteinases (MMP) enzymes thereby increasing the difficulty of cancer cells to invade, e), converting Warburg-type metabolism to mitochondrial oxidative phosphorylation, f), and limiting the ingrowth on new blood and lymph vessels restricting the ability of cancer cells to metastasize. Additional details are available in the text. ET-1, endothelin-1; NF-κB, nuclear factor-κB; OXPHOS, oxidative phosphorylation; VEGF, vascular endothelial growth factor.

metastasis. This change requires disruption of cell:cell and cell:matrix attachments as a result of dissolution of E-cadherin, integrins and other adhering molecules making the cells increasingly mobile, invasive and more resistant to genotoxic treatments (Figure 5). EMT also makes it possible for cancer cells to extravasate into blood and lymphatic vessels and disseminate to remote sites. The molecular events associated with EMT involve a number of transcription factors (ZEB family, Snail, Slug, Twist) and also remodeling of the cytoskeleton [81]. Other signaling pathways involved in EMT include NF-κB, AP-1, Notch, Hedgehog, and Wnt. Transcription factors such as Snail and others often constitute the cargoes of exosomes, one of several types of nanosized vesicles that are released by both normal and, more abundantly, by cancer cells [82]. Exosomes play important functions in the degradation and remodeling of the extracellular matrix, making it easier for cancer cells to invade adjacent tissues.

Melatonin has proven highly effective in limiting EMT and thereby also curtailing metastasis. Metastatic tumor sites typically cause a higher degree of mortality than do primary tumors; thus, restricting the dissemination of cancer cells is of paramount importance since tumors are only diagnosed after they have been initiated and are already progressing. Melatonin interferes with many molecular events required for EMT. It prevents changes in basal to apical polarity of tumor cells, and upregulates adhesion molecules such as E-cadherin and β1-integrin, restricting their detachment from the matrix and from adjacent cells [83]. Suppressing E-cadherin is also important since it normally upregulates numerous factors after it binds to β-catenin; β-catenin aids in the nuclear transcription of the EMT transcription factors, Snail and Slug (Figure 5). For example, in a mouse model of chronic restraint stress which promotes abdominal metastasis of ovarian cancer cells, melatonin treatment effectively attenuated the abdominal burden by partially inhibiting EMT markers involved in the AKT/β-catenin/Slug axis [84]. Concurrently, the cytoskeleton, which determines cellular polarity and contractual forces that allow movement, is reconfigured in cancer cells undergoing EMT to allow for improved migration. Melatonin resists cytoskeletal changes by inhibiting kinases, ROCK1 (Rho-associated kinase 1) and MLCK (myosin light chain kinase) [85], and interfering with changes in vimentin, a major cytosol intermediate filament (Figure 5). Additional actions of melatonin that impeded EMT included interference with NF-κB signaling [86] and direct or indirect downregulation of the classic transcription factors such as Slug, Snail, and Twist. Which of these actions are receptor independent and which are receptor mediated has not been established [87].

To assist with the invasion of cancer cells into surrounding tissues and vascular/lymphatic channels, cancer cells secrete matrix metalloproteinases (MMP-9, −2, −7) that digest collagen and non-collagen fibers and proteoglycans that constitute the extracellular matrix to facilitate their migration. In a variety of different microenvironments, including the protein network encircling cancer cells, melatonin prevents the MMP enzyme-mediated breakdown of the matrix [88], making the invasion of the tumor cells into the surrounding tissue more difficult (Figure 5).

A consequence of EMT and the related molecular perturbations is the improved likelihood for extravasation of the neoplastic cells into blood and lymph vessels. To ensure the availability of blood vessels, tumor cells promote the ingrowth of new branches on the vessels that already exist in the vicinity, a process referred to as angiogenesis. Among the various pro-angiogenic factors released by cancer cells, vascular endothelial growth factor (VEGF) is one of the most important [89]. It induces endothelial cells to sprout, organize into tubules and coalesce with vessels that already exist. The tumor cells then extravasate into the newly formed vessels allowing them to metastasize to remote organs (Figure 5). Melatonin inhibits VEGF in experimental tumor models by modulating hypoxia-inducible factor-1α (HIF-1α) and is associated with reduction in VEGF secretion in advanced cancer patients. Several mechanisms have been proposed to explain melatonin-mediated inhibition of HIF-1α-induced transcription. These include altering the activity of the von Hippel-Lindau protein, a ubiquitin ligase, changing the redox homeostasis of the cell, activating MT1 receptor, or altering microRNAs that impact HIF [90–92].

Endothelin-1 (ET-1), also called preproendothelin-1, is secreted by endothelial cells and upregulates VEGF which normally aids in tumor angiogenesis. Melatonin effectively reduced ET-1 expression by mechanisms that involved downregulation of FoxO1 and NF-κB in colon cancer cells, pointing to an additional process by which it limits the ingrowth of blood vessels in enlarging tumors [81].

6. Melatonin reverses cancer cell chemoresistance

Chemoresistance is a major obstacle in the successful treatment of many cancer types. One feature that relates to the sensitivity or insensitivity of cancer cells to drug treatment is their heterogeneity; this aspect allows some cells to resist the killing effects of chemotherapies while others are vulnerable to the same treatment paradigm. The mechanisms by which cancer cells circumvent or overcome the tumor cell cytotoxicity of chemotherapeutic agents are seemingly multiple, including high catabolism of xenobiotics, increased production of growth factors and DNA repair capacity, enhanced efflux of drugs, gene mutations, and epigenetic alterations [93]. First, to effectively inhibit cancer growth the drug must gain access to the tumor cells in sufficient amounts; in some cases, the unique features of a tumor microenvironment prevent this from happening. When DNA repair processes are enhanced, they can counteract the efficacy of the chemotherapy. Likewise, adjustments of the cell cycle and a reduction in apoptosis contribute to cancer cell resistance as does the activation of signal transduction pathways related to cell survival. The efficiency of autophagy, the status of blood supply, and the tumor microenvironment also impact the degree of resistance to a specific chemotherapy. Drugs that change the redox status of the cancer cell have also been shown to have efficacy in reversing chemoresistance. Finally, the presence of a stem cell-like phenotype allows them to avoid the cell-killing potential of chemotherapeutic agents. Thus, any drug that

reverses tumor insensitivity presumably acts via one or all of these mechanisms, but in essentially all cases the specifics of drug sensitivity-reversal remain unknown [94].

To the authors' knowledge, the first report documenting the ability of melatonin to overcome *in vivo* cancer chemoresistance is that by Dauchy and colleagues [95]. In their study, the authors subcutaneously implanted estrogen receptor-positive (ERα+) MCF-7 human breast cancer cells into immunocompromised nude rats; thereafter, the xenografts were measured daily to determine their rate of growth. In rats kept under constant light, which prevented the nighttime rise in circulating melatonin, the tumors initiated their growth and development more rapidly than those in the animals maintained under a 12:12 light:dark environment with a confirmed circadian melatonin rhythm. When the tumors were sufficiently large, attempts were made to inhibit their further growth by treating the animals with an anti-estrogenic agent, tamoxifen. The breast tumors in the rats maintained under a 12:12 cycle rapidly collapsed, i.e. they responded to chemotherapy. In contrast, the tumors in rats lacking a nighttime rise in melatonin were completely unresponsive to tamoxifen treatment such that they exhibited continued rapid growth. In a further extension of the study, the authors provided rats that lacked endogenous melatonin by adding it to their drinking fluid; this quickly reversed the insensitivity of the tumors to tamoxifen such that their growth was inhibited. Thus, melatonin clearly overcame chemoresistance. At the morphological level, apoptosis, autophagy and reduced cellular proliferation were apparent in the tumors treated with tamoxifen and melatonin, but beyond that, the specific molecular mechanisms of cancer inhibition and chemoresistance were not identified. Obviously, the findings of Dauchy et al [95], indicate that light at night which suppresses the melatonin rhythm may be a risk factor not only for accelerating breast cancer growth but also for rendering them resistant to tamoxifen. This obviously has important implications for the treatment of women with breast cancer using tamoxifen who live in areas where nocturnal light pollution is prominent, e.g. in many cities, or for those who do night shift work [76]. Likewise, the data probably has direct applications to elderly humans as melatonin rhythm is naturally severely dampened in the aged, presumably making them increasingly susceptible to not only cancer initiation but also to tumor chemoresistance and to metastasis [96].

As an example of the importance of these findings, Xiang and colleagues [97] reported that the exposure of breast cancer tumor-bearing rats exposed to light-at-night, which prevents the nocturnal rise in blood melatonin levels, also caused cancer cells to become unresponsive to paclitaxel treatment; however, adding supplemental melatonin reestablished the sensitivity of the cancer to paclitaxel. The authors believe these findings have implications for the treatment of human cancers since the nocturnal environment, where light pollution generally inhibits melatonin secretion and enhances the frequency of cancer [97], makes them less likely to be inhibited by chemotherapeutic drugs.

Doxorubicin (DOX; adriamycin), an anthracycline antibiotic, is a prototypical anti-cancer agent which is widely used in the treatment of a myriad of cancer phenotypes. This chemotherapy has a variety of intracellular targets which determine its efficacy in killing cancer cells, among which is the generation of ROS [98]; as with many other chemotherapies, tumor cells eventually become resistant to doxorubicin. The first report that noted the ability of melatonin to reduce tumor resistance to DOX was published using cultured human hepatocellular carcinoma cells (HepG2 and SMMNC-7721 cells) [99]. In this study, endoplasmic reticulum (ER) stress-mediated chemoresistance was induced using tunicamycin; concurrent treatment of the cells with melatonin reversed the insensitivity of the cells to DOX. Some of the molecular changes that occurred included down-regulation of the PI3K/AKT pathway, elevation of the levels of C/EBP-homologous protein (CHOP) and decreased expression of survivin, a protein of the *Inhibitor of Apoptosis* (IAP) family. Hepatocellular carcinoma is a highly common malignancy that commonly develops drug resistance; if melatonin reverses chemoresistance induced by other stressors, it could drastically improve the treatment of this deadly cancer. Also using HepG2 cells that were resistant to DOX, Hamed and colleagues [100] recently confirmed the ability of melatonin to reverse the failure of DOX to inhibit cancer cells *in vitro*. ATP-binding cassette (ABC) efflux transporters determine the intracellular bioavailability of a drug, xenobiotic, etc. In their study, Hamed et al. [100], showed that genes commonly involved in chemoresistance such as ABCB1, ABCC1, ABCC5 and ABCG2, were inhibited, while caspases −3 and −7, NRF2 and p53 genes were highly expressed because of melatonin exposure. Alvarez-Fernandez and colleagues [101] extended these findings and documented that the ABCG2 receptor plays a central role in determining the biodistribution of not only melatonin but some of its metabolites as well. Xiang and colleagues [102] contributed in vivo data when they observed that resistance of ERα+ MCF-7 tumors to DOX was reversed when the animals bearing the tumors were housed under conditions that allowed them to express elevated melatonin levels at night.

Also using a hepatocellular carcinoma model, Lee and colleagues [103] provided the most complete mechanistic information to explain the resistance of these cells to DOX. For these studies, the authors developed a chemo-resistant hepatocellular carcinoma cell line, named R-HepG2; these cells had a phenotype characteristic of cancer stem cells (CSC). They also expressed high levels of P-glycoprotein (P-gp), a marker of CSC, which ensured easy drug efflux and significantly enhanced their survival capacity. Many cancer cells undergo metabolic reprogramming and adopt a glycolytic phenotype (Warburg-type glucose metabolism) for cytosolic ATP synthesis. Unlike the parent cells from which they were derived

(HepG2), the R-HepG2 cells were not switched to a Warburg-type metabolism. Moreover, they exhibited greatly increased tolerance to DOX, i.e. they were chemo-resistant, with a significant reduction in intracellular concentration of the drug. Also, the drug efflux by P-gp was the result of mitochondrial ATP production (as opposed to glycolytic ATP) and was associated with glutamine metabolism, as blockade of glutamine reduced the sensitivity of R-HepG2 cells to DOX. Thus, the mechanisms of chemoresistance required an altered metabolic reprogramming phenotype which changed drug efflux into R-HepG2 cells (CSC phenotype). This research showed that chemo-resistant cancer cells were relatively glucose independent and used an alternate metabolite, glutamine, to maintain ATP required for DOX resistance; R-HepG2 cells were interpreted to be in a quiescent state [103]. Glutamine is an abundant circulating amino acid with essential roles in cancer cells including those described here for ATP synthesis and also for the production of enzyme antioxidants to maintain redox homeostasis [104]. Relative to the topic of the current review, although not measured in the study [103], presumably mitochondria in R-HepG2 cells maintained high concentrations of melatonin, a potent radical scavenger, to aid in controlling the oxidizing environment of these organelles; high levels of melatonin in the mitochondria would likely protect hepatic cancer cells from DOX toxicity [98].

Cisplatin is a DNA damaging chemotherapeutic agent commonly used to treat highly invasive nasopharyngeal carcinoma (NPC); as with other chemotherapies, however, these tumors often become resistance to the killing effects of cisplatin. Zhang and coworkers [105] explored whether melatonin would reduce cisplatin chemo-resistance as with DOX. They reported that in both *in vitro* studies using NPC cells and *in vivo* investigations that involved the use of an orthotopic NPC xenograft mouse model, melatonin reversed the chemo-resistance to cisplatin and, moreover, even without cisplatin given concurrently, it substantially reduced the growth of NPC. These effects were associated with suppression of the Wnt/β-catenin signaling pathway. Based on these findings [105], the authors advocate the combined use of melatonin in the treatment of NPC.

A fluoropyrimidine-based drug, 5-fluorouracil (5-FU), is a primary chemotherapy used to treat colorectal cancer. The cancer inhibitory actions of 5-FU are exerted primarily by its suppression of thymidylate synthase (TYMS); this enzyme induces the synthesis of thymidylate, a necessary precursor of DNA. As previously described for other such medications, acquired resistance is a feature of persistent 5-FU use; therefore, reversing resistance is pivotal for improving the use of this drug to combat colorectal cancer. Sakatani and colleagues [106] utilized eight human colon cancer cell lines to examine the ability to melatonin to overcome the resistance of these cells to 5-FU. Melatonin not only reversed 5-FU resistance via a mechanism involving the downregulation of TYMS and augmented expression of miR-215-5p, but markedly improved the cytotoxicity of 5-FU when the drugs were used in combination. Additionally, melatonin by itself stymied colorectal cell growth and enhanced apoptosis. With experimental evidence, the authors endorsed the combined use of melatonin in combination with 5-FU as a strategy to treat advanced colorectal cancer patients.

7. Conclusions

Globally, an estimated 10 million people die each year from cancer. While cancer deaths have declined over the last 3 decades, progress has been agonizingly slow considering the financial investment earmarked for finding cures. In the US, 1 of 2 females and 1 of 3 males who develop cancer die as a result of the disease. There are also marked racial disparities in the percentage of people who develop and die of cancer. Cancer is most frequently associated with aged individuals with 9 of 10 cases diagnosed occurring in people over 45 years of age. The frequency of death from metastatic cancer is significantly higher than those who die of primary cancer. Even cancer survivors have residual consequences resulting from the cancer itself or from the medical treatment instituted to inhibit tumor growth.

Melatonin is an endogenously produced, functionally multifaceted molecule that has intrinsic oncostatic activity. In light of the slow progress that is being made in controlling cancer growth and improving survival, it is imperative that all reasonable treatment options be exploited. Experimental, and a modicum of clinical data have overwhelmingly indicated the potential of melatonin in negatively impacting several aspects of tumorigenesis. Coupled with its very limited toxicity even at extremely high doses, its consideration for its extensive testing in clinical trials and experimental use as a treatment for cancer is merited.

The fact that melatonin induces oxidative stress in tumors which contributes to their death, reverses the metabolic profile of tumors, impedes epithelial to mesenchymal transition and overcomes chemo and/or radio-therapeutic resistance emphasizes the importance and urgency of studies that attempt to establish melatonin as a routine treatment/co-treatment for cancer. Also, of noteworthy importance is that one major action of melatonin is to reduce metastases which, when it occurs, is a worst-case scenario for cancer patients. Finally, the elevated frequency of cancer diagnosis in the aged should be considered as endogenous melatonin levels drop dramatically in many people as they age; the loss of this intrinsic anti-tumor agent may be associated with the elevated frequency of cancer onset or the prophylactic use of melatonin may defer tumor initiation and development.

Judging from the sale of melatonin, the number of individuals using this medication, e.g. for sleep, on a daily basis has markedly increased over the last three years;it is assumed this trend will continue for the foreseeable future. It is likely that, in most cases, melatonin is being used by individuals later in life. As a result, a well-controlled epidemiological investigation related to the frequency of specific or all cancer types when compared to individuals who have never used this medication is in order, considering the duration of usage, frequency of use and dosage of melatonin. It is imperative that the large number of potential benefits of this endogenously-produced, non-toxic molecule be utilized for the benefit of humankind. Not to do so seems both unethical and unscientific.

8. Expert opinion

Many of the findings summarized in this review have, unfortunately, been ignored at the clinical level. As discussed in the current review, melatonin has extensive experimental support as a multifunctional anti-cancer agent, particularly in reference to its ability to interfere with the invasiveness and metastatic properties of tumors. These observations have high relevance, considering that metastatic cancer is generally a more serious situation than is a primary cancer. It is the opinion of the authors that the findings have not been seriously considered and tested by the pharmaceutical industry as well as not being introduced to practicing physicians since melatonin, as a natural endogenously-produced molecule is not patentable *per se*, and is uncommonly inexpensive to produce even at the required pharmacological purity for human use. Thus, the financial gain from the use of this agent is much lower than that of prescription medications. Coupled with the essential lack of toxicity of melatonin over an extremely wide dose range, especially relative to that of most oncostatic agents currently in use, it is imperative that clinical trials that either test melatonin alone or in combination with conventional chemotherapies to suppress cancer progression and metastasis is supported. The synergistic actions as a pro-oxidant in tumor cells as well as its ability to rewire cellular metabolism, which provides tumor cells with rapidly generated ATP and an accelerated propensity to produce the necessary molecular building blocks for the enlarging biomass, clearly make it a valuable oncostatic agent. Additionally, melatonin impedes epithelial-to-mesenchymal transition and interferes with the growing tumor's ability to acquire nutrients and metastasize by restricting angiogenesis. The most used anti-cancer agents also have side effects that are substantial and are characteristically diminished by concurrent melatonin treatment. Even if the only benefit of concurrent melatonin administration would be a reduction in collateral toxicity, this would be important for the long-term welfare of patients. Moreover, some chemotherapies are dose-limited because of their side effects; reduction of these side-effects by melatonin may allow higher doses of the anti-cancer drug to be used with greater cancer-killing potential. The chemo- and/or radio-resistance that eventually accompanies the use of many anticancer agents is also a serious issue. The published experimental findings clearly show that for the chemotherapies tested, melatonin either prevents or reverses cancer insensitivity to anti-cancer drugs and radiotherapy. Extensive and expensive efforts are being made to identify molecules that interfere with cancer drug insensitivity. Since melatonin has been shown to have this capacity in animal studies, it is essential that this information be used in the design of human clinical trials. Cancer has long plagued the human population and although treatments have shown steady improvement in cancer outcomes, there is great room for improvement. The authors suggest one molecule, melatonin, as a very viable option for testing. Finally, since artificial light at night suppresses endogenous melatonin production and secretion, cancer patients should be educated about the dangers of random light exposure at night, and lighting in healthcare facilities should be reconfigured to reduce nighttime light exposure.

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