REVIEW
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Melatonin in Mitochondria: Mitigating Clear and Present Dangers

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In cancer cells, glucose is primarily metabolized to pyruvate and then to lactate in the cytosol. By allowing the conversion of pyruvate to acetyl-CoA in mitochondria, melatonin reprograms glucose metabolism in cancer cells to a normal cell phenotype. Acetyl-CoA in the mitochondria also serves as a necessary co-factor for the rate-limiting enzyme in melatonin synthesis, thus ensuring melatonin production in mitochondria of normal cells.

Warburg effect; oxidative phosphorylation; lactate metabolism; pyruvate dehydrogenase kinase; pyruvate dehydrogenase complex; oxidative stress; free radicals; reactive oxygen species

Introduction

Malfunctioning mitochondria contribute to a multitude of disorders referred to as "mitochondrial diseases" (11, 17, 37). Some of the most noteworthy examples include Parkinsonism, cardiomyopathy, diabetes mellitus, cancer, etc. Additionally, many other conditions, not generally categorized in this group, also involve serious dysfunction of mitochondria, e.g., Alzheimer's disease, heavy metal toxicity, ischemia/reperfusion injury, ionizing, and ultraviolet radiation (37, 43, 48, 61).

Mitochondria have many metabolic responsibilities that make their optimal function critical for cell survival. In normal cells, mitochondria account for energy (ATP) production, which results from glucose metabolism (glycolysis) and cellular respiration (oxidative phosphorylation or OXPHOS) in the inner mitochondrial membrane. Glycolysis, which occurs in the cytosol, generates pyruvate, which is actively transported into the mitochondrial matrix. Here, pyruvate is converted to acetyl-CoA, the latter linking glycolysis with the citric acid cycle in the mitochondrial matrix and thus coupling it to ATP production (5, 38). Acetyl-CoA is also an essential co-factor for *N*-acetyltransferase (AANAT), which converts serotonin to *N*-acetylserotonin, the precursor of melatonin (23); AANAT activity rate limits melatonin synthesis (30).

In contrast to normal cells, many solid tumor cells allow the metabolism of glucose to pyruvate in the cytosol but restricts the transfer of pyruvate into the mitochondria; this is known as the Warburg effect (34, 59). In the cytosol of cancer cells, pyruvate is metabolized to lactate, which is abundantly released by these tumors. In addition to this metabolic perturbation, cancer cells invoke processes that ensure rapid cytosolic ATP production as well as the synthesis of macromolecules required for cell division (25). The Warburg effect allows cancer cells to rapidly proliferate, avoid apoptosis, and enhance the invasiveness and metastatic processes characteristic of tumors. Since pyruvate is prevented from forming acetyl-CoA in the mitochondrial matrix of cancer cells, the activity of the citric acid cycle is compromised, OXPHOS is slowed, and ROS generation is reduced; these changes are beneficial to the survival of cancer cells and the growth of tumors.

Herein, we summarize the means by which melatonin reduces mitochondrial irregularities resulting from excessive oxidative stress or as a consequence of the transformation of normal cells to the cancerous phenotype as well its role in mitochondrial dynamics. Also, we summarize the evidence that melatonin has ready access to and is also synthesized in mitochondria. These features put melatonin in the optimal location to counteract oxidative stress and to reprogram the faulty glucose metabolism of cancer cells from the cytosol to the mitochondria. The findings have obvious implications for combating mitochondria-related diseases and for curtailing cancer cell growth and metastasis.

Mitochondria: A Mother Lode of ROS

The electron transport chain (ETC), which supports OXPHOS in the inner mitochondrial membrane, consists of a series of proteins that pass electrons between successive carriers from high-energy molecules to lower-energy acceptor proteins. When an electron chemically reduces the

next carrier in the sequence, the energy that is produced aids in pumping hydrogen atoms from the matrix across the inner mitochondrial membrane to the intermembrane space. This creates an electrochemical gradient across the inner mitochondrial membrane that is used by ATP synthase to generate ATP (9).

The passage of electrons between successive carriers in the ETC is not flawless, with some electrons being misdirected and then interacting with neighboring ground-state oxygen molecules (O_2) to produce ROS, e.g., the superoxide anion radical ($O_2^{\cdot -}$) (8, 12, 29). The $O_2^{\cdot -}$ is the first in a chain (FIGURE 1) of partially reduced oxygen derivatives that destroy adjacent molecules, including those of the ETC. Damage to the carrier molecules in this system contributes to greater inefficiency such that more electrons are fumbled, leading to ever-increasing numbers of ROS. To inhibit ROS-mediated molecular and functional impairment, ROS must be neutralized before they affect essential mitochondrial elements. This action is left to a series of enzymes that either metabolize ROS to innocuous species or to a sequence of molecules that directly scavenge them (18, 44). These agents are collectively referred to as antioxidants and include melatonin (52, 66). The damage that ROS creates is known as oxidative stress and generally compromises the functions of all cells.

Melatonin: A Firewall Against Oxidative Stress

The persistent battle against corrosive oxygen-based reactants in organisms is carried out by radical scavengers and antioxidant enzymes. The oxidizing processes make a significant contribution to cellular and organ decay and, therefore, to organismal diseases and aging (8, 51). Numerous radical scavengers have evolved to fight the battle waged by the oxygen derivatives. One of the earliest evolved of these scavengers is believed to have been melatonin. This low-molecular weight derivative of tryptophan came into being an estimated 3 billion years ago when life on Earth consisted of prokaryotic bacteria, including photosynthetic cyanobacteria (33, 39, 53, 65). These organisms persist to the present day. Although some early prokaryotes were not concerned about ROS since atmospheric oxygen levels at that time were near zero, the fact that cyanobacteria were photosynthetic caused them to generate O_2 and a large number of oxygen-based reactants. The released O_2 eventually caused atmospheric O_2 to increase to present-day levels. This made the need for an effective radical scavenger a high priority, thus the presumed evolution of melatonin (53, 65). Melatonin functions as an antioxidant in plants as it does in animals (7, 28).

In addition to its high efficiency as a combatant against oxidative stress, throughout a very long evolutionary process, melatonin has been repurposed to perform other critical subcellular tasks without a modification of its original chemical structure (73). Some of these metabolic jobs include its participation in circadian/circannual rhythm regulation, sleep promotion, anti-inflammation, and perturbations that also reduce chronic diseases (56, 58). Its original function in the conservation of efficient metabolism due to its high radical-scavenging efficiency, however, survives in present-day species (73). Besides its preeminent function in the direct detoxification of radicals and associated non-radical products, its metabolites are also uncommonly good scavengers (16, 57, 66).

Melatonin has taken on the additional responsibility of promoting the activities of antioxidant enzymes while suppressing pro-oxidant enzymes (FIGURE 2) (3, 42, 68). As an example, melatonin augments glutathione levels by stimulating its synthesis (14); glutathione is an important antioxidant that often is present in very high concentrations in cells (74). In mitochondria, melatonin upregulates the activity of a major antioxidant enzyme, superoxide dismutase 2

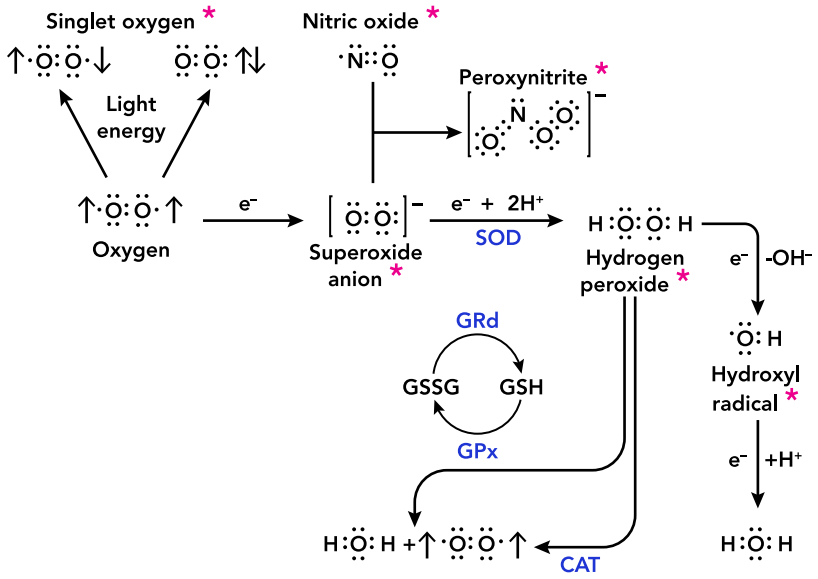

FIGURE 1. **Ground-state oxygen is the initial source of many derivatives, referred to as reactive oxygen species, which are generated in vivo**

Whereas some ROS play important roles in signal transduction, when produced in excess they are highly toxic to neighboring molecules. The ROS marked with an asterisk (*) are those that have been shown to be scavenged by melatonin, with the bulk of these studies having been performed under in vitro or in pure chemical systems. In addition to the scavenging actions of melatonin, it also stimulates antioxidant enzymes, e.g., both cytosolic and mitochondrial superoxide dismutase (SOD), glutathione peroxidase (GPx) and reductase (GRd), and catalase (CAT). Many of these observations derive from in vivo investigations. It is difficult to prove direct radical scavenging in cells, so the percentage of protection provided by direct detoxification of ROS versus that due to stimulation of antioxidant enzymes remains a major unknown. GSH, reduced glutathione; GSSG, oxidized glutathione.

(SOD2), by promoting sirtuin 3 (SIRT3), which deacetylates SOD2, allowing it to carry out its function of dismutating $O_2^{\cdot -}$ to hydrogen peroxide (H_2O_2) (19, 55). Although both SOD2 and glutathione peroxidase (GPx) (FIGURE 1) limit the oxidizing environment of the matrix and intermembrane space (IMS), it has been difficult to determine the relative importance of direct scavenging versus indirect metabolism of the toxic reactants in preserving mitochondria fitness. Considering the multiple means by which melatonin resists oxidative stress, it seems to be a veritable "Swiss Army knife" of the antioxidant family.

At any intracellular site, to effectively neutralize the most destructive ROS, the scavenging molecule must be essentially juxtaposed to the reactive species when it is generated, i.e., it must be in what has been described as the "reaction cage" of the ROS. This is necessary since the half-life of highly reactive ROS are extremely short (for the ·OH, half-life is 10^{-9} s), and they travel infinitesimally short distances before they engage and oxidize a neighboring healthy molecule (14). Thus a scavenger does not have time to attack a ROS from afar—it must be on-site. The damage caused by free radicals is frequently described at the point where the radical was generated (32), i.e., site-specific damage.

Mitochondria-targeted antioxidants have taken "center stage" as potential agents to resist oxidative stress, which contributes to a large number of diseases and to tissue/organ deterioration during aging (13, 27, 49, 51, 60). Both small-molecule, free-radical scavengers and ROS-metabolizing enzymes are being considered as highly useful agents in combatting molecular damage to not only mitochondria but in other cellular compartments as well. Melatonin is cataloged as a small molecule,

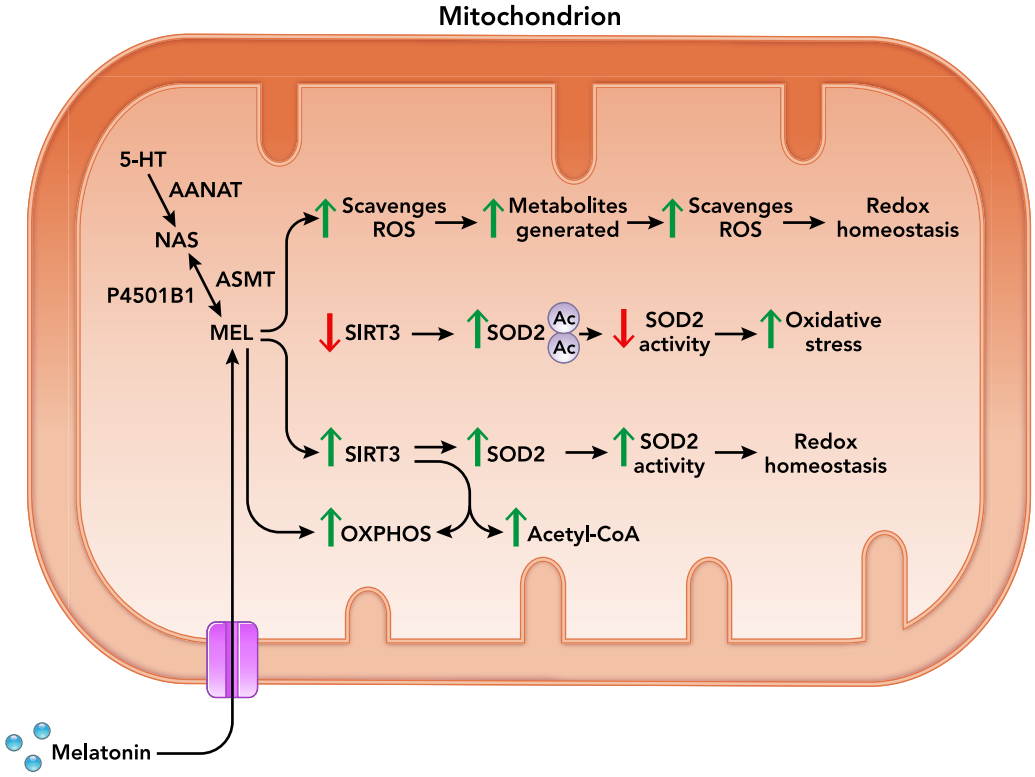

FIGURE 2. **Epigenetic regulation of mitochondrial SIRT3 by melatonin and its association with the redox state of this organelle**

In normal cells, mitochondria contain high concentrations of melatonin, presumably via its uptake through the oligopeptide transporters PEPT1/2, and due to its intramitochondrial synthesis. Melatonin effectively incapacitates ROS, likely via electron donation and other means, resulting in the generation of metabolites that are also ROS scavengers; these actions aid in the maintenance of the redox homeostasis in the mitochondria (*top* pathway). The antioxidative enzyme, superoxide dismutase 2 (SOD2), is also essential for reducing the oxidizing environment in mitochondria. When SIRT3 is downregulated (*middle* pathway), SOD2 is acetylated and its activity is depressed, increasing the likelihood of ROS-mediated oxidative damage. Melatonin upregulates SIRT3, leading to the deacetylation of SOD2 and an increase in the activity of the dismutating enzyme, which helps to maintain a reduced redox environment in mitochondria (*bottom* pathway). SIRT3 has other actions in mitochondria, including the improvement of OXPHOS; this action is shared by melatonin, possibly due to its stimulation of SIRT3. Upregulation of SIRT3 also leads to the accumulation of acetyl-coenzyme A (acetyl-CoA), which is a co-factor for the rate-limiting enzyme in melatonin synthesis, AANAT. Red arrows show decreased activity; green arrows show increased activity. 5-HT, serotonin; AANAT, arylalkylamine *N*-acetyltransferase; ASMT, acetylserotonin O-methyltransferase; NS, *N*-acetylserotonin; MEL, melatonin; OXPHOS, oxidative phosphorylation; P_{450} 1B1, cytochrome P_{450} 1B1 enzyme (it reverses metabolized MEL to NAS).

mitochondria-targeted agent (20, 53) with both direct ROS scavenging functions and an ability to elevate antioxidant enzyme activities (15, 72). Because of this categorization, Lowes and coworkers (35) compared the efficacy of melatonin to synthetically produced MitoE or MitoQ to forestall inflammation and oxidative mutilation in animals subjected to severe oxidative stress, i.e., treatment with two bacterial toxins that are highly destructive, lipopolysaccharide and peptidoglycan. Because of their high lipophilicity, MitoE and MitoQ concentrate up to 1,000-fold over their parent molecules in mitochondria (27). Melatonin is in naturally high concentrations in this organelle (69). When the animals were treated with equimolar amounts of each of the three antioxidants, all suppressed the inflammatory response and the number of oxidized molecules to different degrees. When the data were critically evaluated, however, the conclusion was that, of the three anti-inflammatory/antioxidant agents administered, melatonin would be the most useful in overcoming mitochondrial dysfunction (35). Melatonin may be superior since not only it but several of its metabolites likewise neutralize ROS, thereby multiplying its efficiency in limiting molecular injury. Since the molecular decimation inflicted by ROS is often described as being site specific, it has been suggested the melatonin may have a positional advantage for scavenging. Melatonin's high concentration in mitochondria (69) as well as its rapid uptake from the blood (1) suggest this could be the case, but beyond that there are no findings that support this assumption.

Melatonin in Mitochondria: Home Sweet Home

For years, melatonin was known to have a major impact on mitochondrial function as manifested by its ability to improve the efficiency of the electron transport chain (40), enhance ATP production (2), and reduce mitochondrial ROS damage (26, 46, 47). These findings suggested that melatonin may actually be in this organelle. With the aim of determining whether this is the case and whether non-pineal cells exhibit a 24-h rhythm in melatonin levels consistent with those in the blood, Venegas et al. (69) collected rat brain and liver samples over a 24-h light-dark cycle and compared melatonin levels in cell membranes, mitochondria, cytosol, and nuclei. Although none of the organelles exhibited reliable circadian variations in melatonin levels, there were slight fluctuations in successively collected tissue samples that were likely attributable to differential utilization/metabolism of the indole. There were, however, large differences in the levels of melatonin at different sites with mitochondrial values greatly exceeding (up to 10-fold) those in other subcellular organelles. Importantly, mitochondrial melatonin levels were clearly not related to pineal-derived circulating melatonin concentrations, since the concentrations were not depressed in rats that had been pinealectomized for several weeks in which blood melatonin levels are near zero. Since the pineal gland was not the origin of the melatonin measured in the brain and liver samples, it was speculated that the cells may synthesize their own melatonin, an idea not totally inconsistent with earlier reports that had measured melatonin in many non-pineal tissues.

The persistently low concentrations of melatonin in the blood after pinealectomy is interesting in view of the apparent widespread synthesis of melatonin in non-pineal tissue. Clearly, melatonin from extra-pineal sites does not normally have access to the vascular system. The mechanisms that exclude non-pineal sources from releasing melatonin into the blood are unknown but may relate to some unique morphological features of the pineal gland, e.g., the presence of canaliculi, which are not shared by most other tissues.

Melatonin has many beneficial actions on the peripheral reproductive organs, which have been especially well-defined in the female. The concentrations of melatonin in the preovulatory ovarian follicular fluid are higher than in the blood (45), and adding melatonin to medium containing recently ovulated or vitrified oocytes aids their healthy maturation and development and improves pregnancy outcome (50, 64). To explain the marked effects of melatonin on the multiple crucial changes the oocyte undergoes after its discharge from the ovary, He and colleagues (21) considered the possibility that oocytes, as had been presumed for other cells (69), may produce their own melatonin. When mouse oocytes were immunocytochemically examined for the presence of AANAT, it was found and localized in oocyte mitochondria and was present at all stages of oocyte maturation (21). When fresh oocytes were incubated in a medium containing serotonin, an essential precursor of melatonin, the melatonin concentrations in the oocytes and in the incubation medium rose substantially; this did not occur in oocytes deprived of serotonin. Although control of pinealocyte melatonin synthesis had been thoroughly investigated (31), this pathway had not previously been attributed to the mitochondria.

Besides strongly indicating that oocyte mitochondria synthesize their own melatonin, the findings have additional long-term implications in both female and male mammals. The mitochondria in the cells of adult mammalian species are of maternal origin. If oocyte/zygote mitochondria retained the

capacity to generate melatonin during implantation, gestation, and post-delivery development, it would be expected that the cells of all adult mammals would continue to have the capability to generate melatonin. The retention of the melatonin synthetic capacity of mitochondria in all species has been proposed (53, 65).

The strongest evidence that supports mitochondrial synthesis of melatonin is that of Suofu et al. (63). With the use of mouse brain non-synaptosomal mitochondria, the two enzymes required for melatonin synthesis from serotonin, i.e., AANAT and ASMT, as well as the chaperone 14-3-3ζ were co-localized to the mitochondrial matrix (neuronal and glial). Similar to Venegas et al. (69), Suofu and co-workers found that mitochondrial melatonin levels did not exhibit a day-/night-mediated circadian rhythm. Furthermore, they found that the knockout of AANAT, which rate-limits melatonin synthesis, led to increased oxidative damage in response to processes that elevated ROS production. Finally, when mitochondria were incubated with deuterated serotonin, they produced high concentrations of deuterated melatonin and its metabolites (63).

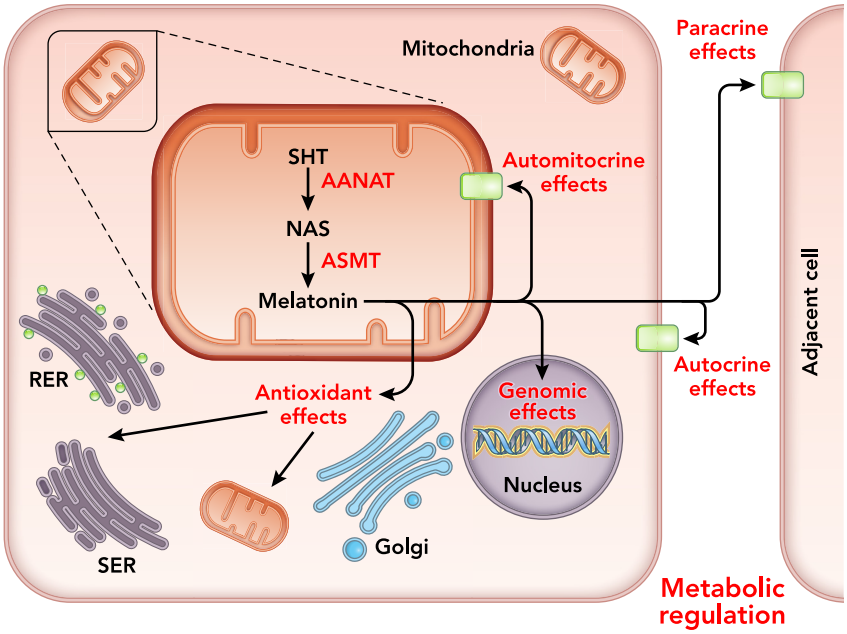

FIGURE 3. **Evidence suggests that, of all cells, mitochondria produce melatonin**
Unlike the pinealocytes, which release melatonin into the cerebrospinal fluid and blood, mitochondria-synthesized melatonin in non-pineal cells is used locally in metabolic regulation and is not released into the systemic circulation. Although surgical pinealectomy causes persistently barely detectable concentrations of melatonin in the blood, it has no impact on the much greater concentrations of melatonin in mitochondria. Following its synthesis in mitochondria, melatonin, in addition to its functions in this organelle, diffuses out of the mitochondria, where it feeds back onto melatonin receptors in the mitochondrial membrane (the automitocrine actions) as well as impacting the genome. Melatonin also likely diffuses into the intracellular space where it exerts receptor-mediated autocrine and paracrine actions. AANAT, arylalkylamine *N*-acetyltransferase; ASMT, acetylserotonin methyltransferase.

Since they had previously localized the MT1 melatonin receptor on the mitochondrial membrane (70) (an observation confirmed in the later report), Suofu et al. (63) proposed that melatonin is released from mitochondria, after which it acts via the MT1 receptor to control cytochrome *c* release, a process the authors refer to as the automitocrine regulation (FIGURE 3). Soon after their report, Ahluwahia (4) published a supporting letter that immunocytochemically identified both of the MT1 and the MT2 receptors on the mitochondrial membranes of gastric endothelial cells. Their illustrations suggest a very high density of melatonin receptors on these mitochondria.

In addition to its synthesis in this organelle, evidence is strong that melatonin can also be taken into cells from the blood (26, 46, 47). How melatonin is transported into cells and into mitochondria has recently been investigated. The studies of Hevia et al. (22) indicated that neither the intracellular concentrations of melatonin nor the kinetics of the uptake were consistent with a passive diffusion model of melatonin uptake. Rather, its entrance into cells was deduced to require a protein-mediated process, with the uptake being closely related to the glucose concentration of the medium. The involvement of such a means for melatonin uptake was supported by docking studies and showed that the protein transporter was likely GLUT1. Although the results of Hevia et al. (22) are compelling, the rate of the uptake of melatonin was slower than anticipated and may be inconsistent with some of the data related to the published actions of melatonin. In both in vitro and in vivo conditions, the addition of melatonin quenches intracellular and intramitochondrial oxidative stress usually within minutes (26, 46, 47).

An alternative active transport system for the entrance of melatonin into cells and into mitochondria was described by Huo et al. (24). They determined that the human oligopeptide transporters PEPT1/2 are involved in mediating the movement of melatonin into cells and, importantly for the current review, into mitochondria. Using multiple cancer cell types, this group reported that melatonin is a substrate for the PEPT1/2 transporters. Docking studies confirmed the binding of melatonin to the transporters, and the authors noted its suitable binding configuration for the receptor protein. Moreover, the transporters were located in the mitochondrial membrane and found to accelerate melatonin uptake into this organelle. Since a major interest of Huo et al. (24) was the anticancer actions of melatonin, normal cells were not examined for the presence of the PEPT1/2 transporters. The authors predicted that the tumor-inhibiting actions of melatonin required its uptake into mitochondria.

Having melatonin in the mitochondria resulting from its uptake and de novo synthesis provides functional advantages to these organelles and to cells as a whole. During OXPHOS, ROS are abundantly formed, and melatonin, as an indiscriminate radical scavenger (67) (FIGURE 1), is in an ideal position to incapacitate these damaging brigands. The reader is reminded that free radicals, in particular those that are most reactive, decimate critical molecules in the immediate neighborhood of where they are generated. Having an agent such as melatonin, which preys on free radicals in the mitochondria, provides an opportunity to prevent them from initiating damaging chain reactions where multiple crippled macromolecules are the result. Melatonin is a veritable "fox in the hen house."

Melatonin: Interactions With Mitochondrial Glucose Metabolism

Blask et al. (10) reported that human mammary cancer cells growing in vivo released large amounts of lactate during the day but markedly lower levels at night. Similar measurements of other parameters (glucose uptake, DNA synthesis, and DNA content) of the tumors fluctuated accordingly. The findings suggest that the cancers exhibited the Warburg effect (cytosolic glycolysis) during the day but not at night and that the cells were more similar to the cancer cell phenotype during the day while they were of a more normal phenotype at night. Suppressing the nocturnal rise in circulating melatonin with nighttime light exposure converted the tumors to Warburg-type metabolism continuously over the 24-h period. This showed that the more normal cell phenotype of the cancers at night was related to the nighttime rise in melatonin since circulating nocturnal melatonin concentrations are inhibited by nighttime light exposure.

In normal cells after glucose uptake into the cytosol, it undergoes glycolysis to pyruvate. Thereafter, the bulk of the pyruvate is translocated into the mitochondria, where it is enzymatically converted to acetyl-CoA by pyruvate dehydrogenase complex (PDC). Acetyl-CoA is a critical molecule in mitochondria since it feeds the citric acid cycle, which eventually ensures optimal ATP production by OXPHOS (FIGURE 4). In many cancer cells that exhibit the Warburg effect, pyruvate uptake by mitochondria is severely blunted, since PDC, the enzyme that metabolizes it to acetyl-CoA, is inhibited by the gatekeeper enzyme, pyruvate dehydrogenase kinase (PDK), which is upregulated in many cancer cells (62). As a result, pyruvate is metabolized to lactate in the cytosol, which is then released into the circulation (36).

The results of Blask and colleagues (10), however, showed that lactate release from human breast cancer cells growing in vivo was elevated only during the day. Moreover, the nocturnal switch in glucose metabolism to the mitochondria was governed by the nighttime rise in endogenous melatonin levels, since its inhibition prevented the switch.

We speculate that circulating nighttime melatonin levels enter the mitochondria, where they inhibit PDK, allowing for the upregulation of PDC (54) (FIGURE 4). The reprogramming of glucose metabolism and overcoming the Warburg effect by melatonin may explain the cancer-inhibiting actions of this indole, since it deprives cancer cells of the rapid production of ATP in the cytosol and also reduces the synthesis of the abundant macromolecules required for the rapid cell proliferation, e.g., via the pentose phosphate shunt. The inhibition of PDK is a common mechanism by which other molecules redirect glucose metabolism in cancer cells and suppress tumor growth (62).

Mitochondrial acetyl-CoA, the product of pyruvate metabolism, has another critical function in this organelle. Acetyl-CoA is known to be a necessary co-factor/substrate for the rate-limiting enzyme in melatonin synthesis AANAT (31) (FIGURE 4). Thus the conversion of pyruvate to acetyl-CoA in normal cell mitochondria ensures endogenous melatonin synthesis in these organelles (54). Due to inhibition of PDC in cancer cells, acetyl-CoA is not available to assist with the production of melatonin since AANAT is not supported by the co-factor. To date, all measures of melatonin concentrations and its synthesis have been performed using normal (non-cancerous) cells. We predict that melatonin synthesis does not occur in cancer cell mitochondria because of the deficiency of acetyl-CoA (54). If, however, nocturnal circulating melatonin levels are sufficiently elevated, it does enter the mitochondria to inhibit PDK, allowing for the nocturnal switch away from the Warburg effect, as observed by Blask et al. (10).

In addition to the proposal described to explain how melatonin may redirect glucose metabolism in cancer cells (54), Mayo and co-workers (41) have likewise deduced that melatonin has a significant impact on glucose uptake and metabolism in cancer cells. These schemes require thorough experimental analysis, and the processes described likely relate to the efficacy of melatonin in modulating the Warburg effect.

In mitochondria, melatonin is converted back to its precursor *N*-acetylserotonin (NAS), when it is *O*-demethylated by CP450 1B1 (71) (FIGURE 4). Thus NAS may also be a critical factor in maintaining optimal mitochondrial metabolism. In fact,

inhibition of CP450 1B1 reduces the levels of NAS and interferes with the anticancer actions of melatonin, whereas overexpressing the enzyme has the opposite effects (71). The equilibrium between melatonin and NAS is established by both ASMT and CP450 1B1, with the activities of these two enzymes being differentially regulated between normal and cancer cells (67). We have recently provided a comprehensive explanation of the possible importance of the NAS-to-melatonin ratio in determining the growth and inhibition of the highly deadly tumor glioblastoma multiforme (6). The ideas advanced, however, may well apply to many tumors, and the NAS-to-melatonin ratio could become a critical target for anticancer therapy.

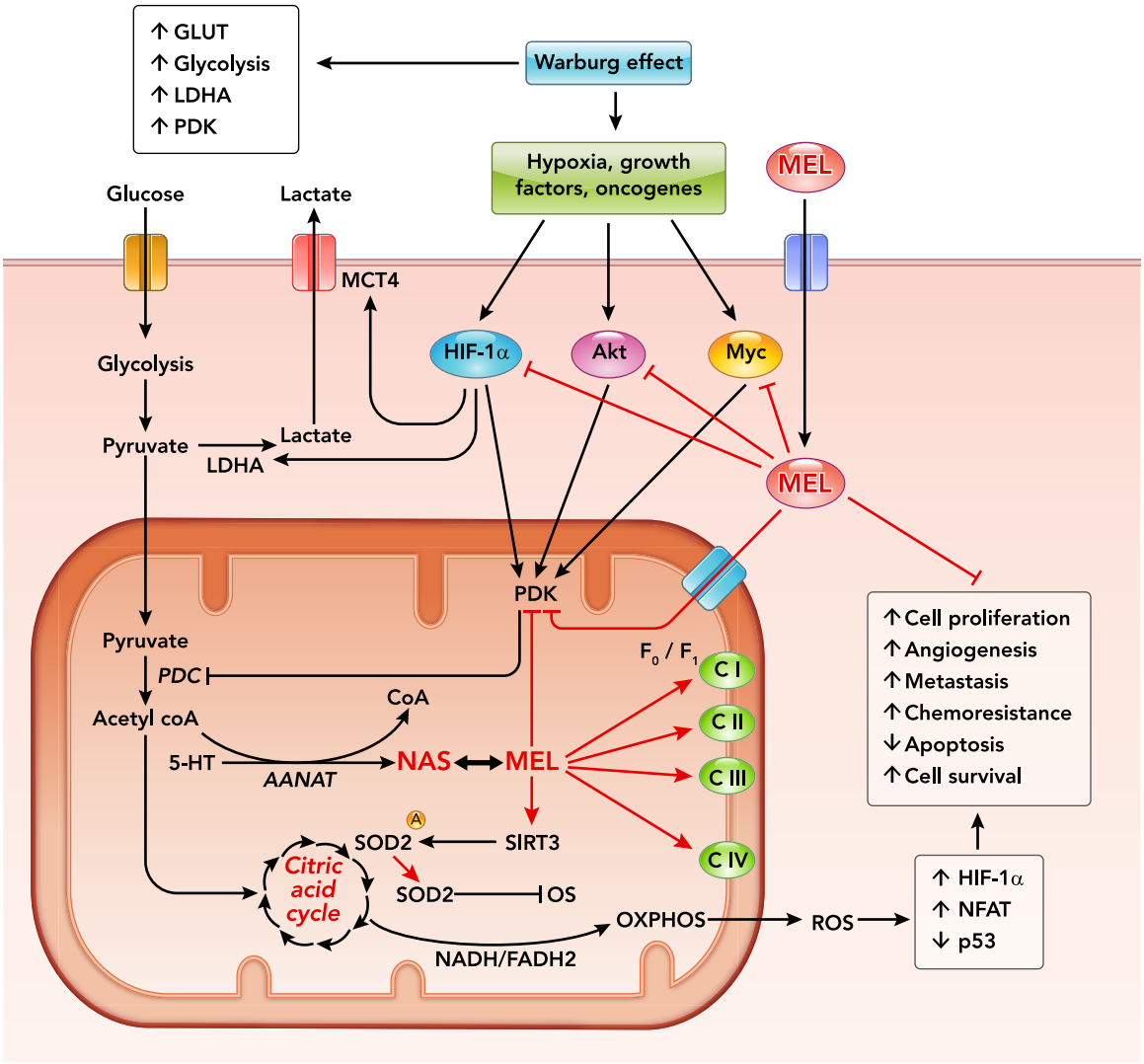

FIGURE 4. **A summary of the processes involving the functional interactions of melatonin with mitochondrial physiology in normal and cancerous cells**

In normal cells, glucose enters the cytosol via the glucose transporter (GLUT), where it is metabolized to pyruvate, the bulk of which is translocated into the mitochondria, where, under the action of pyruvate dehydrogenase complex (PDC), it is metabolized to acetyl-CoA. Acetyl-CoA feeds the citric acid cycle, which supports OXPHOS in the inner mitochondrial membrane. Acetyl-CoA is also a required co-factor/substrate for the rate-limiting enzyme in melatonin synthesis, arylalkylamine *N*-acetyltransferase (AANAT), which converts serotonin (5-HT) to the precursor of melatonin, *N*-acetylserotonin (NAS), which is transformed into melatonin. Melatonin can also be reverse-metabolized to NAS (see text for details). Melatonin and NAS directly scavenge partially reduced ROS generated during OXPHOS. Additionally, melatonin stimulates sirtuin 3 (SIRT3), allowing for the deacetylation of superoxide dismutase 2 (SOD2), leading to its activation, thereby reducing oxidative stress (OS). Many solid tumor cells change their handling of pyruvate such that, rather than being shunted into the mitochondria, it is metabolized to lactate with the aid of the enzyme lactate dehydrogenase A (LDHA); lactate is then released in large quantities into the blood. Pyruvate does not enter mitochondria since PDC is inhibited by the gatekeeper enzyme, pyruvate dehydrogenase kinase (PDK), which is upregulated in cancer cells. This combination of metabolic changes that cancer cells undergo is referred to as the Warburg effect and provides tumor cells advantages in terms of tumor biomass enlargement, invasiveness, and metastasis. Also, since acetyl-CoA is not produced in cancer cell mitochondria, they are likely incapable of producing their own melatonin. Bloodborne melatonin, either derived from the pineal gland or after its administration, can enter the cytosol and mitochondria via several means (see text for details), where it presumably inhibits PDK, allowing for the activation of PDC; melatonin thus reverses the Warburg effect and aids in arresting cancer cell growth. Arrows indicate stimulation; blunt lines indicate inhibition. $FADH_2$, flavin adenine dinucleotide; HIF-1α, hypoxia inducible factor 1α; MCT4, monocarboxylate transporter (lactate transporter); NFAT, nuclear factor of activated T cells.

Epilogue

Melatonin has been routinely found to be helpful in combating one of the major shortcomings of OXPHOS, i.e., losing control of electrons as they are shuttled between successive respiratory chain complexes such that they chemically reduce oxygen. This event culminates in the generation of a variety of ROS, some of which inflict damage, oxidative stress, on neighboring molecules. This molecular debilitation is often a prime obstacle to the proper function of mitochondria as well as other subcellular processes and represents a major danger to cells and organs.

Since melatonin has been shown to be highly effective in battling against oxidative stress, its uptake by and proposed synthesis in mitochondria puts it in the optimal location to protect against the ROS onslaught. When an overwhelming (pharmacological) number of radicals are generated, physiological levels of melatonin are incapable of preventing all the damage. Under these conditions, only pharmacological amounts of the antioxidant can combat oxidative stress.

The ability of melatonin to manipulate glucose metabolism by shifting it primarily from the cytosol to the mitochondria may be a critical mechanism that accounts for its oncostatic actions in cancers that exhibit the Warburg effect. The mechanisms to hypothetically explain how melatonin manages this reprogramming of glucose metabolism are elaborated herein. Other agents that inhibit cancer, e.g., dichloroacetate, are believed to reverse the Warburg effect by the same mechanism as proposed here for melatonin.

Melatonin exists in two pools in vertebrates. Blood melatonin levels are maintained by that produced in and released from the pineal gland. The mitochondrial pool is not discharged into the circulation but rather is used by the cells (or adjacent cells) that produce it. The melatonin synthesized in the pineal gland is seemingly $<5\%$ of the total melatonin produced.

Excessive oxidative stress in any cell and the transformation of normal cells into a cancer phenotype qualify as clear and present dangers for cells, organs, and organisms. Melatonin is highly efficient at reducing oxidative stress and seems to prevent the conversion of mitochondrial OXPHOS of normal cells to aerobic glycolysis (the Warburg effect) of cancer cells. ■

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R.J.R., Q.M., and R.S. prepared figures; R.J.R. drafted manuscript; R.J.R. and R.S. edited and revised manuscript; R.J.R., Q.M., and R.S. approved final version of manuscript.

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