

Review

Inhibition of mitochondrial pyruvate dehydrogenase kinase: a proposed mechanism by which melatonin causes cancer cells to overcome cytosolic glycolysis, reduce tumor biomass and reverse insensitivity to chemotherapy

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ABSTRACT

This review presents a hypothesis to explain the role of melatonin in regulating glucose metabolism in cancer cells. Many cancer cells use cytosolic glycolysis (the Warburg effect) to produce energy (ATP). Under these conditions, glucose is primarily converted to lactate which is released into the blood in large quantities. The Warburg effect gives cancer cells advantages in terms of enhanced macromolecule synthesis required for accelerated cellular proliferation, reduced cellular apoptosis which enhances tumor biomass and a greater likelihood of metastasis. Based on available data, high circulating melatonin levels at night serve as a signal for breast cancer cells to switch from cytosolic glycolysis to mitochondrial glucose oxidation and oxidative phosphorylation for ATP production. In this situation, melatonin promotes the synthesis of acetyl-CoA from pyruvate; we speculate that melatonin does this by inhibiting the mitochondrial enzyme pyruvate dehydrogenase kinase (PDK) which normally inhibits pyruvate dehydrogenase complex (PDC), the enzyme that controls the pyruvate to acetyl-CoA conversion. Acetyl-CoA has several important functions in the mitochondria; it feeds into the citric acid cycle which improves oxidative phosphorylation and, additionally, it is a necessary co-factor for the rate limiting enzyme, arylalkylamine N-acetyltransferase, in mitochondrial melatonin synthesis. When breast cancer cells are using cytosolic glycolysis (during the day) they are of the cancer phenotype; at night when they are using mitochondria to produce ATP via oxidative phosphorylation, they have a normal cell phenotype. If this day:night difference in tumor cell metabolism is common in other cancers, it indicates that these tumor cells are only cancerous part of the time. We also speculate that high nighttime melatonin levels also reverse the insensitivity of tumors to chemotherapy.

Key words: Oxidative phosphorylation, N-acetyltransferase, pyruvate kinase, acetyl-CoA, aerobic glycolysis chemotherapy, melatonin.

1. INTRODUCTION

Several metabolic features of cancer cells are unique. For example, many solid tumor cells abandon mitochondrial glucose oxidation in favor of cytosol-based glycolysis even under conditions of normoxia (1, 2); this metabolic switch is known as the Warburg effect (3, 4). Normally, the glucose metabolite, pyruvate, is moved from the cytosol into the mitochondrion due to the activity of pyruvate translocase (PTL) where it is converted to acetyl-CoA by pyruvate dehydrogenase complex (PDC). PDC is under control of the “gatekeeper” enzyme pyruvate dehydrogenase kinase (PDK). PDC is inactivated due to its reversible phosphorylation by PDK using ATP as a phosphate donor (7). Down regulation of PDC results in a shutdown of mitochondrial glucose oxidation and accelerates cytosolic aerobic glycolysis as a source of ATP, such as occurs in cancer cells (8, 9). Deactivated PDC is re-activated under the influence of pyruvate dehydrogenase phosphatase (PDP) (10).

Regulation of the PDC/PDK axis is considered a potential means for cancer inhibition (8, 9, 11). Dichloroacetate (DCA), a mitochondria-targeted molecule and an inhibitor of PDK (9, 12), causes the upregulation of PDC which aids in the transport of pyruvate into the mitochondrial matrix where it is irreversibly converted to acetyl-CoA which feeds the citric acid cycle (6). In doing so, DCA reduces aerobic glycolysis and inhibits cancer growth (13).

Melatonin, a secretory product of the mammalian pineal gland (14, 15), has well known oncostatic properties (16, 17). The nocturnal rise in melatonin causes experimental breast tumors to switch from aerobic glycolysis to mitochondrial glucose oxidation (18). When the nocturnal melatonin rise is prevented by exposing cancer-bearing animals to light at night, the tumors do not switch to mitochondrial glucose oxidation but rather persistently metabolize glucose to lactate in the cytosol (18). This failure allows the tumors to grow faster than those in animals maintained under a light:dark cycle where the night is accompanied by high melatonin levels. The inhibitory effects of melatonin on cancer growth are similar to those of DCA.

Herein, we proposed that melatonin, like DCA, reduces cancer cell growth by inhibiting PDK thereby activating PDC to reduce aerobic glycolysis and tumor cell proliferation. Also, like DCA, melatonin is targeted to the mitochondria (19, 20).

2. MITOCHONDRIAL UPTAKE AND SYNTHESIS OF MELATONIN

The transformation of normal cells to cancer cells often causes mitochondria to limit their uptake of pyruvate. This metabolite is then made available for its conversion to lactate in the cytosol with the production of ATP, i.e., it allows for the development of cytosolic aerobic glycolysis (Warburg effect); this occurs in many solid tumor cells. This switch is impacted by melatonin. This is readily apparent in cancer-bearing rats when they are experimentally deprived of a nocturnal melatonin surge; in that case, the tumors produce lactate at a persistently high rate (18) (Fig. 1). Conversely, when blood melatonin levels are elevated due to nighttime darkness or as a result of melatonin supplementation, it is taken up by the tumor cell mitochondria (21-23) where it causes cancer cells to no longer secrete large amounts of lactate because they have switched to mitochondrial glucose metabolism and ATP production.

If melatonin influences mitochondrial metabolism as indicated above, it presumably enters this organelle. More than a decade ago, Jou *et al.* (24, 25) initially reported that free radicals in mitochondria are quickly immunochemically quenched when melatonin is added to the incubation medium. Melatonin and its metabolites are well-known direct scavengers of toxic

oxygen derivatives, so its ability to reduce immunocytochemically-detected reactive oxygen species was not surprising (26, 27). The findings also showed, however, the likelihood that melatonin had ready access to the mitochondria. Even prior to the first publication of Jou *et al.* (24), melatonin was shown to enhance mitochondrial ATP synthesis suggesting it may enter this subcellular organelle (28).

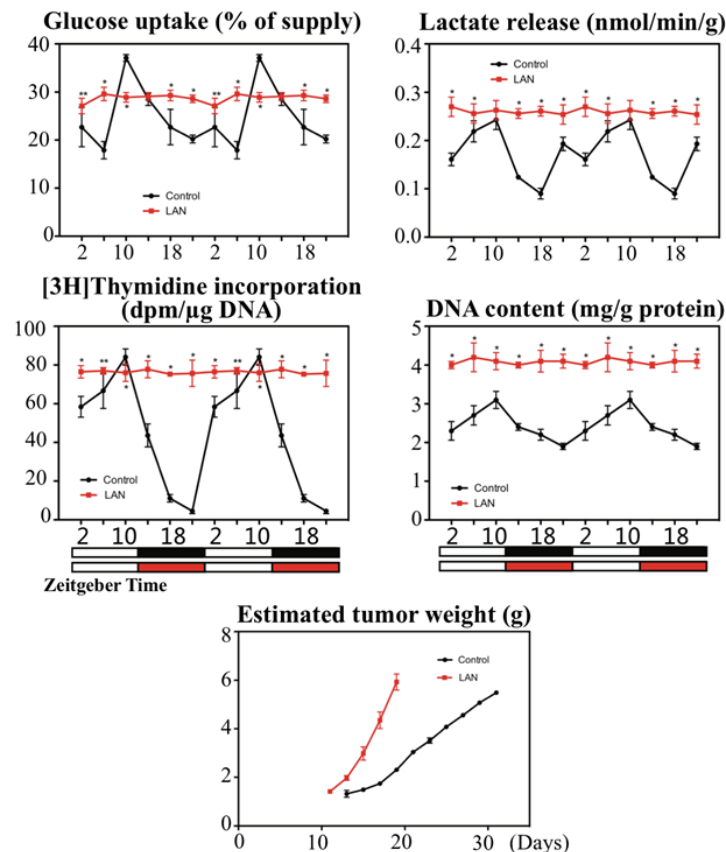


Fig. 1. Glucose uptake, lactate release, thymidine incorporation into DNA and total DNA of xenografted breast cancer cells growing in immunocompromised rats.

The high lactate secretion identifies periods when the cells are using cytosolic glycolysis for ATP production. Under normal light:dark cycles (horizontal bar at bottom with alternating white and black segments), glucose metabolism to lactate and parameters of DNA synthesis were high during the day and low at night (black curves). This signifies that during the day (with low blood melatonin) the tumors function under conditions of cytosolic glycolysis (Warburg effect) but at night, when melatonin levels in the blood are elevated, the tumors were using mitochondrial oxidative phosphorylation for ATP production; however, when the dark period was contaminated with light (alternating white and red bar at bottom) at night, which prevented the nighttime rise in blood melatonin, cytosolic glycolysis and DNA parameters were persistently high (red curves). The panel at the bottom illustrates the differential tumor growth in animals kept in a normal light:dark cycle (black curve) and in animals exposed to light at night (LAN) (red curve). Figure drawn based on the data of Blask *et al.* (18).

While these early studies did not estimate the actual concentrations of melatonin in mitochondria, when these values were finally measured, they were found to be much higher (up to 10x higher) than in other subcellular organelles and very significantly elevated over those in the blood (29). One possibility to explain the high melatonin concentrations in the mitochondria was that mitochondria may actively take up melatonin from the blood against a concentration gradient. This explanation was dismissed, however, when it was found that high mitochondrial melatonin concentrations were not diminished even after long-term pinealectomy (29), which causes circulating levels of the indole to be near zero.

More recent detailed studies specifically designed to determine how melatonin is taken up by cells and mitochondria have been published (29). Due to its high lipid solubility, it was initially assumed that melatonin may merely enter the cell/mitochondria by passive diffusion. While this possibility is not totally discounted, other active uptake processes have been described. According to Hevia *et al.* (30, 31), melatonin's entrance into cancer cells (nine cancer cell types were studied) requires a protein-mediated process and that the uptake of melatonin is highly influenced by the glucose concentration of the incubation medium. The authors eventually determined that melatonin enters cancer cells through the GLUT1 transporter. The studies, however, were not extended to examine melatonin transport into mitochondria.

Also using cancer cells, Huo *et al.* (32) conducted a more detailed analysis of melatonin's route into mitochondria; they reported that this was accomplished via human oligopeptide transporters, PEPT1/2. Docking studies of melatonin showed the indoleamine is a match to the unity of the transporters; they were likewise strongly correlated with melatonin transfer from the cytosol into the mitochondrial matrix.

Both Hevia *et al.* (30) and Huo and coworkers (32) specifically investigated the transfer of melatonin from the extracellular space into the intracellular environment using cancer cells. This being the case, both groups predicted that melatonin, likely related to its receptor-independent processes, inhibits cancer cell proliferation; however, they provided no details of what other molecular events may be involved. Also, there are receptors/binding sites inside of cells on which melatonin could have acted, e.g., quinone reductase 2 (MT3) (33), the reported receptors in the mitochondrial membrane (MT1/MT2) (34) and nuclear receptors (35). Especially the findings of Huo *et al.* (32) put melatonin in the position where it could directly influence both PDC and PDK activities.

For decades, many investigators had noted the close functional relationship between melatonin and mitochondrial pathophysiology (36, 37). Also following the discovery of melatonin in a bacterium (38) this idea was further advanced. According to the theory of endosymbiosis (39), mitochondria (and chloroplasts) of all current eukaryotes evolved from bacteria that were phagocytized as food by early eukaryotes. Since bacteria have been found to possess (38) and, more recently discovered, to possibly synthesize melatonin (39), we (40) speculated that mitochondria (and chloroplasts) of all extinct and living eukaryotes (animal and plant) produce melatonin using the mechanisms inherited from their ancestral prokaryotic bacteria.

The validity of this hypothesis has been greatly strengthened by the published reports documenting melatonin's production, in part or in total, by mitochondria (34, 41-44) and by chloroplasts (45, 46). In a report that was almost totally ignored by the scientific community, Kerenyi and colleagues (41) provided evidence that pinealocyte mitochondria immunocytochemically expressed the rate limiting enzyme in melatonin synthesis, arylalkylamine N-acetyltransferase (AANAT), both in the mitochondrial matrix and in the

intermembrane space. The immunoreactive product was only apparent when acetyl-CoA was added to the mixture; acetyl-CoA is a necessary co-factor for the acetylating enzyme, which determines the amount of melatonin produced.

Mouse oocyte mitochondria (42), and in a subsequent publication, mitochondria from 4-cell stage, 8-cell stage zygotes and blastocysts (43), also exhibited immunoreactive AANAT. Moreover, when isolated mitochondria were incubated with serotonin, a necessary precursor of melatonin, concentrations of melatonin rose in both the mitochondria and incubation medium. Mitochondria recovered from these early-stage zygotes also exhibited reduced melatonin-synthetic activity when AANAT was knocked down with siRNA. Finally, non-symptosomal mitochondria from mouse brain were found to contain both the AANAT and ASMT proteins and, in the presence of deuterated serotonin, the mitochondria generated deuterated melatonin and melatonin-related products (34).

While the studies summarized all claim the melatonin-synthetic pathway is, at least in part, confined to the mitochondria, they differ in terms of AANAT submitochondrial localization. The study by Kerenyi *et al.* (41) suggests the presence of immunoreactive AANAT in both the mitochondrial matrix and in the intermembrane space. The results of Suofu and colleagues (34) suggested that the bulk of the AANAT was in the matrix while He *et al.* (42) believe it to be primarily in the intermembrane space. None of the studies are sufficiently definitive to prove that the entire synthetic pathway is restricted to the mitochondria. Mitochondria do, however, synthesize melatonin and these organelles contain high concentrations of this indoleamine (23, 29).

As with the uptake of melatonin, its production in the mitochondria gives it ready access to the metabolic machinery that controls the enzymes that mediate the conversion of pyruvate to acetyl-CoA in these organelles. Besides potentially governing the movement of pyruvate into the mitochondria, the presence of melatonin in these organelles has other important cellular, organ, and organismal actions as summarized in a number of reviews (47-53).

3. AEROBIC GLYCOLYSIS (WARBURG EFFECT) OF CANCER CELLS

The Warburg effect, i.e., the metabolic shift from mitochondrial oxidative phosphorylation to cytosolic glycolysis in tumor cells is for the benefit of the proliferating cancer cells and detrimental to the cancer-bearing host (Figure 2). It is a maneuver cancer cells have evolved to promote aggressive cellular proliferation and enhanced long-term maintenance. Also, tumor cells undergoing frequent mitoses require an abundant energy supply to support their rapid growth. Under those circumstances, intermediates produced during glycolysis provide the necessary biomolecules. For example, glucose-6-phosphate can enter the pentose phosphate pathway with the synthesis of ribose-5-phosphate and 3-carbon molecules, essential for lipid and nucleic acid synthesis (54). Finally, this metabolic route aids in maintaining redox homeostasis. NADPH produced via the pentose phosphate pathway serves as a reducing agent which is particularly important during the high ROS load produced under conditions of very active cellular growth (55).

Inhibition of glycolysis has emerged as a potential means of restraining cancer growth. Since cancer cells downplay mitochondrial function suggests that the specific physiology of these organelles has antitumor actions. So “forcing” cancer cells to use mitochondrial oxidative phosphorylation and associated metabolic processes may be a means of inhibiting cancer cell proliferation/growth and overriding tumor resistance to chemotherapy. Indeed, glycolysis has

become a site of attack to test oncostatic agents which are glycolytic inhibitors: 2-deoxyglucose, 3-bromopyruvate, and the pharmaceutical drug, Lonidamine (56, 57). While each of these has shown some efficacy in cancer inhibition, there is concern regarding potential untoward side effects or toxic reactions (58).

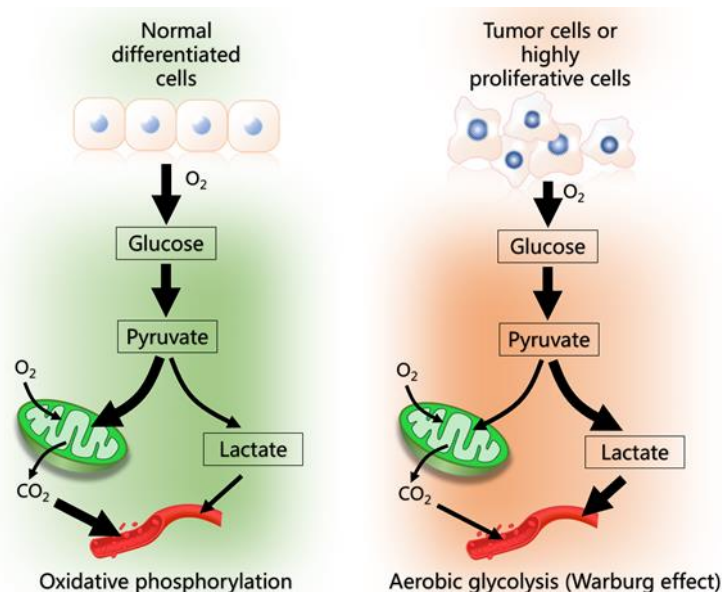


Fig. 2. Differences of glucose metabolism between normal and cancer cells.

In contrast to normal cells, cancer cells abandon mitochondrial oxidative phosphorylation in favor of cytosolic glycolysis for ATP production. This gives cancer cells an advantage in terms of the synthesis of macromolecules required for rapid cellular proliferation, cellular growth and the avoidance of apoptosis. It also renders them insensitive to chemotherapeutics. Cancer cells metabolize pyruvate to lactate which is released into the blood. The mitochondria of normal cells take up pyruvate where it is converted to acetyl-CoA which feeds the citric acid cycle, improves ATP production and promotes intramitochondrial melatonin synthesis by serving as a necessary co-factor for the rate limiting enzyme in melatonin synthesis (AANAT).

Dichloroacetate (DCA), like melatonin, also is a glycolysis inhibitor (13) that is considered a potential anti-cancer agent and for its ability to reverse cancer chemoresistance (59). DCA promotes the conversion of pyruvate to acetyl-CoA in mitochondria at the expense of cytosolic glycolysis. Mechanistically, DCA inhibits PDK to unleash PDC which converts pyruvate to acetyl-CoA in mitochondria where it feeds the citric acid cycle and elevates oxidative phosphorylation (60, 61). This ensures greater superoxide anion radical production by the electron transport chain, reduces cell proliferation, and enhances apoptosis.

Since melatonin is present, although seemingly not exhibiting a day:night rhythm in mitochondria (34), it continually ensures the conversion of pyruvate to acetyl-CoA in this organelle by indirectly stimulating PDC. PDC is activated since we theorize that melatonin inhibits PDK, which normally suppresses PDC. Thus, like some other agents, e.g., DCA, melatonin is a glycolytic mimetic which switches cells from using cytosol glycolysis to oxidative phosphorylation for ATP production. This aids cells that were previously cancerous in developing a more normal phenotype.

As already noted, in the mitochondrial matrix, pyruvate is converted to acetyl-CoA, a multifunctional molecule. Among other actions, acetyl-CoA promotes the citric acid cycle when the two-carbon acetyl group undergoes a condensation reaction with the four-carbon oxaloacetate to form citrate. Also, in the mitochondria, acetyl-CoA, as in the pineal gland, serves as an essential co-factor for AANAT, the rate limiting enzyme in melatonin production (62). The melatonin formed then inhibits PDK activity, allowing the continual conversion of pyruvate and acetyl-CoA by removing the inhibitory effect of PDK on PDC (Figure 3). Thus, melatonin supports its own synthesis.

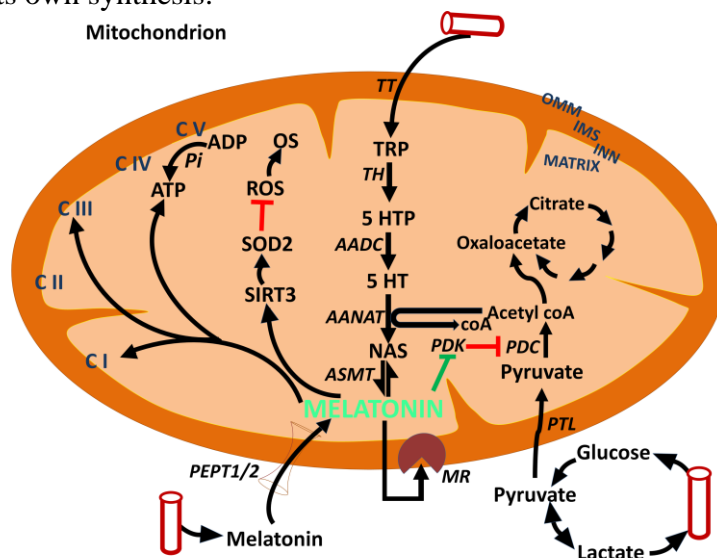


Fig. 3. The proposed mechanisms by which melatonin switches cancer cell glucose metabolism from cytosolic aerobic glycolysis (lower right) to mitochondrial oxidative phosphorylation.

In doing so, melatonin reduces cell proliferation, increases apoptosis, limits tumor biomass accumulation and curtails metastases. In tumor cells, melatonin, which can be taken into mitochondria via the oligopeptide transporters (PEPT1/2) as well as synthesized in these organelles, inhibits pyruvate dehydrogenase kinase (PDK) allowing the upregulation of pyruvate dehydrogenase complex (PDC) which metabolizes pyruvate to acetyl-CoA. Acetyl-CoA is known to enter the citric acid cycle by coupling with oxaloacetate. Additionally, acetyl-CoA allows intramitochondrial melatonin production since it is a critical co-factor for the rate limiting enzyme in melatonin synthesis, arylalkylamine N-acetyltransferase (AANAT). Melatonin has other actions in mitochondria (not all illustrated in this figure) including improving the efficiency of the electron transport chain (CI-CIV) and increasing ATP synthesis. Melatonin also regulates mitochondrial oxidative homeostasis by activating SIRT3 to deacetylate superoxide dismutase 2 (SOD2) which then metabolizes ROS to innocuous molecules. In the absence of melatonin, cancer cells utilize cytosolic aerobic glycolysis to produce energy: in this case, glucose is primarily converted to lactate. AADC = aromatic acid decarboxylase; ADP = adenosine diphosphate; ASMT = acetylserotonin methyltransferase; IMM = inner mitochondrial membrane; IMS = intermembrane space; MR = melatonin receptor; NAS = N-acetylserotonin; OMM = outer mitochondrial membrane; OS = oxidative stress; Pi = inorganic phosphate; PTL = pyruvate translocase; TH = tryptophan hydroxylase; TRP = tryptophan; TT = tryptophan transporter; 5-HT = serotonin; 5-HTP = 5-hydroxytryptophan.

As mentioned, we predict that the mitochondria of cancer cells lose their ability to produce melatonin while this synthetic pathway remains functional in normal cells. The mechanisms by which the mitochondria of cancer cells shut down melatonin production has yet to be determined. In cancer cells, PDC can only be influenced by circulating melatonin (of pineal origin) which enters the mitochondria. A potentially important corollary of this is that, if the shutdown of melatonin production in cancer cell mitochondria could be prevented, the conversion of normal cells to a cancerous phenotype could possibly be reduced.

There are additional implications of the findings of Blask *et al.* (18) which show that high circulating nighttime melatonin levels switch tumor glucose metabolism from cytosolic glycolysis to mitochondrial oxidative phosphorylation. Because of this issue, cancer in aged individuals should be re-evaluated. In most elderly individuals (63) melatonin levels drop remarkably (presumably at all sites in all cells) such that they have no or a weak circadian melatonin rhythm in the blood and no melatonin synthesis in mitochondria (as in young rats exposed to light at night). Because they are devoid of a nocturnal melatonin rise, tumors would use cytosolic glycolysis 24 hours daily which would allow them to rapidly increase their biomass and their likely metastasis. Considering this, some consideration should be given to the use of melatonin, especially by the older population, for the purpose of slowing tumor growth by reversing the Warburg effect. Melatonin can be given in conjunction with conventional chemotherapies (64-67).

The ability of melatonin to retard/prevent/reverse the insensitivity of tumors to a chemotherapy, e.g., tamoxifen (68), also may relate to its ability to switch tumor cell glucose metabolism from aerobic glycolysis to oxidative phosphorylation. Indeed, when mammary tumors in rats became resistant to tamoxifen, the insensitivity was overcome by daily melatonin administration (68).

The theoretical processes proposed here would explain why Blask and coworkers (18) found that breast cancer xenografts growing in immune-compromised rats exhibited cytosolic glycolysis (high lactate production) during the day and oxidative phosphorylation (low lactate production) (Fig. 1) at night. Circulating melatonin levels are low during the day and high at night. The hypothesis is also consistent with the observations that suppressing nighttime melatonin levels by exposing the animals to light caused the tumors to experience glycolysis and high lactate secretion throughout the 24-hour period. This rhythm of daily alternating periods of glycolysis and oxidative phosphorylation in tumor cells apparently has never been observed for other types of cancers. Such information would be important to know and suggests that individuals with solid cancers should presumably avoid light exposure at night which interferes with the nocturnal surge of melatonin (69, 70). It also indicates that breast cancer cells may be of the cancer phenotype only in the absence of melatonin.

4. CONCLUSION AND PERSPECTIVE

The theoretical suggestions proposed herein are supported by already-published data (71-75) and are consistent with the following predictions: a) melatonin is capable of reprogramming glucose metabolism from cytosolic glycolysis to mitochondrial oxidative phosphorylation; b) melatonin achieves this switch by inhibiting pyruvate dehydrogenase kinase (PDK) activity which allows for the upregulation of pyruvate dehydrogenase complex (PDC) resulting in the conversion of pyruvate to acetyl-CoA; c) acetyl-CoA serves several functions in the mitochondria, i.e., among others it feeds the citric acid cycle and it is a necessary co-factor

for the rate limiting enzyme in melatonin synthesis, AANAT; d) melatonin is in high concentration in the mitochondria of normal cells, where it is synthesized, but absent or in lower levels in cancer cell mitochondria where it is not synthesized; e) by suppressing the Warburg effect in cancer cells, melatonin reduces cancer cell proliferation and metastases; f) this action of melatonin not only reduces the cancer phenotype but also interrupts the insensitivity of cancers to chemotherapies.

There are other issues that deserve consideration regarding the presence and proposed actions of melatonin at the mitochondrial level in cancer cells. As illustrated in figure 1, it shows that high lactate secretion (indicative of cytosolic glycolysis, the Warburg effect) by breast cancer cells occurs during the day when pineal-derived circulating melatonin concentrations are at their nadir and, for the reasons proposed herein, cancer cells are incapable of synthesizing their own melatonin at least during the day. Of special note regarding figure 1, however, is at night lactate secretion is low indicating the cancer cells were no longer engaging in cytosolic aerobic glycolysis. The implication of these very marked differences in day:night lactate release by cancer cells is that these tumors function with a cancer phenotype during the day but at night they are functioning as normal cells. With darkness onset, even in cancer-bearing animals, melatonin is uniquely released from the pineal gland into the circulation. Upon arrival at the cancer site, melatonin is taken into cancer cell mitochondria where it inhibits PDK (which upregulates PDC) allowing the cells to produce acetyl-CoA and convert to a more normal cell phenotype (Fig. 3). Thus, based on the findings summarized herein, the mammary tumor cells in this model were only “part time” cancerous, i.e., during the day.

An extensive search of the literature did not uncover any previous report wherein tumors exhibited such a marked day:night difference in glucose metabolism. Scientists typically investigate cancer cell metabolism from tumors collected during the day when melatonin levels are low. Perhaps other cancer types also utilize cytosolic glycolysis during the day and display a more normal cell phenotype at night. Thus, perhaps most tumors (in animals and humans) living under usual light:dark conditions are only “cancerous” during the day due to the loss of a pineal-derived melatonin signal. If so, this strongly re-emphasizes the importance of maintaining darkness at night since as light pollution at night continues to become worse, pineal melatonin production will be reduced, and associated cancers may continue to increase.

Cancer mechanisms at the molecular level are extremely complex and the definitive processes by which melatonin modulates cancer cell growth have yet to be identified. In this regard, the reverse Warburg effect is currently under intensive investigation as a means of arresting tumor growth since it may be the Achilles’ heel of cancers (76). Based on the observed actions of melatonin, it is expected that melatonin will impact this process as well.

In addition to the means by which melatonin influences cancer growth as hypothesized herein, it has other actions by which it restricts the initiation, progression and metastasis of tumors (17, 77-79). In fact, we have suggested that the major benefit of melatonin in cancer therapy will be in terms of its ability to minimize cancer invasiveness and its migration to secondary sites (80-82). Metastatic movement of cancer is a factor that is also impacted by the failure of these cells to utilize their mitochondria for glucose oxidation in lieu of cytosolic glycolysis.

Other reported actions that have been mentioned as processes used to repress cancer progression include limiting growth factor uptake (83, 84), improving immune surveillance (85, 86), reducing oxidative/nitrosative stress (86, 88), controlling angiogenesis (81, 89), synchronizing circadian rhythms (90-94), etc. Nevertheless, we contend that ensuring pyruvate

metabolism to acetyl-CoA in mitochondria will prove to be of major importance in determining the ability of melatonin counteract tumor growth.

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AUTHORSHIP

RJR conceived the idea and produced an outline. This was then discussed with the co-authors during which time they submitted ideas for inclusion. All co-authors read preliminary and the final versions of the manuscript. RS, QA and SRC collaborated on the production of the figures.

CONFLICT INTEREST

The authors declare no conflicts of interest.

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