Commentary

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Melatonin synthesis in and uptake by mitochondria: implications for diseased cells with dysfunctional mitochondria

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Although there is one exception (red blood cells), the lack of energy (ATP) provided by mitochondrial oxidative phosphorylation (OXPHOS) is not compatible with the long-term survival of normal cells. During conventional metabolism, pyruvate, the cytosolic glycolysis product, enters the mitochondria, where it is metabolized to acetyl-coenzyme A (acetyl-CoA) under the influence of the enzyme pyruvate dehydrogenase complex (PDC). Acetyl-CoA makes an important contribution to the tricarboxylic acid (TCA) cycle, which feeds NADH and $FADH_2$ to the respiratory chain, where they benefit the generation of ATP by OXPHOS.

In some diseased cells, however, pyruvate metabolism becomes aberrant since its transport into the mitochondria is blunted due to the downregulation of PDC because of its inhibition by pyruvate dehydrogenase kinase (PDK), which in turn is upregulated by hypoxia-inducible factor 1α (HIF-1α). Therefore, pyruvate undergoes fermentation to lactate in the cytosol. This alternate pathway of pyruvate metabolism is known as the Warburg effect, named after the individual who discovered it, Otto Warburg. Since pyruvate does not enter the mitochondria, mitochondrial ATP synthesis is depressed. Warburg-type metabolism, however, compensates for this by rapidly, albeit inefficiently, synthesizing ATP in the cytosol. Warburg metabolism (also known as aerobic glycolysis) is almost always associated with pathological cells.

Melatonin in mitochondria: concentration & synthesis

Melatonin (N-acetyl-t-methoxytryptamine) was discovered in a neural outgrowth, the pineal gland, in 1958. For decades thereafter, this methoxyindole was recognized for its circadian (clock) and circannual (calendar) synchronizing actions, such as the sleep–wake cycle and seasonal reproduction. Subsequently, reports were published proving that melatonin is present in many extrapineal tissues [1], and when examined it was identified in mitochondria, where its concentration is much greater than in other subcellular organelles [2]. Importantly, these high levels persisted in tissues of animals that had their pineal gland surgically removed, so the mitochondrial melatonin in peripheral cells was not of pineal origin [2]. Because of its high concentration in mitochondria, it was surmised that it is synthesized in these organelles [3].

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That mitochondria may produce melatonin is consistent with its evolution. Melatonin is an ancient molecule that is believed to have originated as an antioxidant in prokaryotes 3.0–2.5 billion years ago [4]. These bacteria were eventually phagocytosed by the earliest prokaryotes (2.5–1.5 billion years ago), where they evolved into mitochondria (the endosymbiotic theory for the origin of mitochondria). After doing so, the ability of prokaryotes to synthesize melatonin was retained since its relentless antioxidant activity was critical in an organelle such as the mitochondria that produce an abundance of reactive oxygen species (ROS)/free radicals. Not only did the early eukaryotes take advantage of melatonin actions in the mitochondria, every eukaryotic cell that evolved thereafter found it advantageous to retain melatonin synthesis in this organelle.

These findings prompted a search for melatonin-forming enzymes in mitochondria of present-day eukaryotic cells, the outcome of which revealed that indeed these organelles have retained the two enzymes required to convert serotonin to melatonin, such as arylalkylamine N-acetyltransferase (AANAT) and acetyl serotonin methyltransferase (ASMT) [5]. Moreover, when challenged with the appropriate substrates, the mitochondria rapidly synthesized melatonin. Although the Suofu *et al.* [5] study is elegant and thorough, an earlier study by He *et al.* [6] has one feature that makes it of utmost importance. After the isolation of the mitochondria from rodent oocytes, these organelles were found to convert serotonin to melatonin. The critical nature of this lies in the fact that all mitochondria in adult mammals are maternally derived. Thus, we predicted that during embryological development and postnatal growth, mitochondria of every cell retained the melatonin-forming ability because of the advantages it provided as a potent antioxidant in this ROS-rich organelle [7].

Consequences of the absence of melatonin synthesis in mitochondria

The inability of cells to generate melatonin in their mitochondria invariably contributes to cellular disease. A shutdown of mitochondrial melatonin synthesis occurs when the intramitochondrial conversion of pyruvate to acetyl-CoA does not occur [7]. Acetyl-CoA is a necessary co-factor/substrate for the rate-limiting enzyme in melatonin synthesis (AANAT), and without it, melatonin cannot be produced. Cancer cells, as well as other diseased cells, hijack pyruvate metabolism from the mitochondria by preventing its conversion to acetyl-CoA, causing its fermentation to lactate in the cytosol (the Warburg effect). Without acetyl-CoA production in mitochondria, melatonin formation is also halted. Loss of melatonin, a potent antioxidant in mitochondria, has serious consequences in terms of enhanced oxidative stress and diminished ATP production accompanied by elevated ROS/free radical generation [8]. These changes have dire consequences for mitochondrial and cellular physiology and are common in mitochondrial syndrome and mitochondrial diseases.

Melatonin is a critical intramitochondrial antioxidant since it functions as a direct free radical scavenger and by stimulating antioxidant enzymes [4], including superoxide dismutase 2 (SOD2), catalase and glutathione reductase. Each of these enzymes participates in maintaining mitochondrial redox homeostasis. Glucose metabolism and the Warburg effect have been extensively studied in many disease states [9–12]. By necessity, the measurements have always been performed on tissues collected during the day with the assumption that these end points were also the same at night. Several years ago, however, it was reported that experimental mammary tumors (derived from human cancer cells) growing *in vivo* employed Warburg-type metabolism during the day (as had been reported many times), but at night, the tumors switched from Warburg metabolism to mitochondrial OXPHOS based on measurements of glucose utilization and lactate production [13]. Moreover, this remarkable nocturnal transformation was controlled by the pineal-derived nocturnal rise in circulating melatonin. The mitochondria of the cancer cells are presumably incapable of their own melatonin synthesis during the day because they lack the necessary substrate, acetyl-CoA [9]. Recall that the cancer cells cannot produce melatonin because the enzyme that catalyzes pyruvate to acetyl-CoA, PDC, is strongly downregulated due to inhibition by PDK, which is upregulated by HIF-1α [14]. Melatonin has been repeatedly shown to inhibit HIF-1α, resulting in a reduction of PDK activity and the disinhibition of PDC, allowing mitochondrial melatonin synthesis to occur and for OXPHOS to be activated. During hypoxia, HIF-1α regulates several genes that promote cellular adaptation to adverse conditions. The elevated expression of glucose transporters regulated by HIF-1α, in particular GLUT-1 and GLUT-3, increases glucose uptake and enhances glycolysis, which is predominant under hypoxic conditions. Inhibition of HIF-1α, for example by melatonin, leads to the downregulation of glycolysis in cancer cells, thereby altering tumor adaptive mechanisms [15].

During the day, melatonin is neither produced in the mitochondria nor is there any available from the blood, so cancer cells revert back to Warburg-type metabolism [7]. What this implies, of course, is that during the day, cancer cells are functioning as such, but at night they behave with a healthier phenotype – that is, they are only cancerous 50% of the time [8]. It seems likely that when other cancerous and diseased cells are examined at night (when

collected in darkness), they will also be found to exhibit a day–night metabolic shift, thus making the mitochondria dysfunctional only part of the time. This should be documented in other diseases where melatonin is shown to be inhibitory by comparing daytime and night-time glucose metabolism [13]. If similar observations are made in other disease models, such as observed for mammary cancer cell metabolism, at least relative to pyruvate metabolism, it would indicate that they also are only pathological 50% of the time [7]. Such studies cannot be performed *in vitro*, since cells growing in culture are not exposed to a circadian melatonin rhythm. This exposes a shortcoming of some cancer metabolism studies.

Pineal-derived, nocturnally elevated blood melatonin is taken up by mitochondria via the oligopeptide transporters, PEPT 1/2, by diseased cells, where it inhibits HIF-1α, thereby disinhibiting PDC. This stabilizes mitochondrial physiology by allowing them to synthesize melatonin and to use OXPHOS as a means of ATP production, and therefore it reconfigures diseased cells to a more normal phenotype. As a result, it is obvious that anything that interferes with the night-time melatonin rise would also compromise the mitochondrial function of all cells.

Although exogenously administered melatonin inhibits cell proliferation of most cancer cells, there are also a few cancer cell types where it additionally induces cell death. A major factor that accounts for the different responses in the cells where melatonin induces cell death is the unexpected large increase in ROS generation. Several genes have been recently reported to be differentially expressed after melatonin treatment in one of the cell types in which ROS production is exaggerated [16]. One such protein that is clearly expressed is dihydrolipoamide (DLD), an element of the PDC enzyme complex. DLD is downregulated in these tumor cells, and PDC cannot be re-activated even after HIF-1α is inhibited by melatonin. This situation results in reduced intramitochondrial acetyl-CoA synthesis and, consequently, suppression of downstream mitochondrial functions, leading to a massive rise in ROS that causes cell death [16]. In most cancer cells, melatonin reconfigures Warburg metabolism to the more normal oxidative phosphorylation for ATP production. In these exceptional cell types, melatonin also reverses Warburg-type metabolism but does not allow OXPHOS or other mitochondrial functions to occur, causing these cell types to die.

Given that manufactured light sources, which are commonly used after darkness onset in most current societies, can inhibit pineal melatonin production and release, the adoption of an indoor lifestyle with the misuse of light at night both reduces melatonin levels and desynchronizes the regular melatonin rhythm that was common in our evolutionary ancestors who depended on the rising and setting of the sun for their light–dark information. Hence, the often-reported association between light-at-night, melatonin suppression and increased cancer incidence may well be a result of the accompanying perturbed mitochondrial physiology.

There are several unavoidable natural conditions where the night-time melatonin rise is severely blunted. For example, there are individuals who have genetically based melatonin insufficiency, referred to as hypomelatoninemia. Likewise, throughout life, the melatonin rhythm becomes dampened such that in many elderly individuals, this cycle almost disappears due to the failure of the pineal gland to produce and secrete melatonin nightly. A loss of melatonin at the mitochondrial level also accompanies aging [17]. Thus, aged individuals would be expected to have significantly perturbed mitochondrial function, which they do [18]. As a corollary, cellular mitochondria are essentially in a permanently 'diseased state' such that aged individuals display an increased vulnerability to cancer development (and other mitochondria-related diseases). An excellent treatment to help in improving mitochondrial physiology in late life may be the night-time use of melatonin by these individuals, a solution that has been suggested by many scientists.

Melatonin & the metabolic shift

In addition to the report showing that melatonin mediates the metabolic shift from Warburg metabolism to OXPHOS in mammary cancer [13], there are other examples of disease states where cellular metabolism can be forced to fluctuate between these metabolic phenotypes. The conversion of hyperinflammatory M1 macrophages to less inflammatory M2 macrophages also depends on this metabolic shift, an action that is likely mediated by melatonin [19]. Disease states that exhibit abnormal pyruvate metabolism are classified as mitochondrial diseases or that they suffer from the mitochondrial syndrome. There is a long list of such conditions [20]. In diseases where the metabolism of cells could be transformed to the more conventional OXPHOS, the severity of these conditions would likely be reduced. There is an intensive ongoing search for molecules (called glycolytics) that can reconfigure Warburg-type metabolism to OXPHOS, the best known of which is dichloroacetate (DCA). DCA is a synthetic molecule which, like endogenously produced melatonin, is an anticancer agent. Both DCA and melatonin are inhibitors of HIF-1α, which is important for maintaining a more normal mitochondrial phenotype.

Conclusion & perspective

Due to the uncommonly large number of disease processes that melatonin influences, we recently suggested that these conditions may have a yet-to-be-identified common basis that melatonin impacts [7]. Here, we propose that this common denominator likely involves melatonin synthesis and its actions in mitochondria. Mitochondrial dysregulation including aerobic glycolysis (Warburg) is linked to a plethora of varied pathophysiological conditions such as cancer, psychological illnesses, inflammation, fibrosis, Alzheimer disease, traumatic brain injury, and autoimmunity. It will be the goal of future scientists to define the association of melatonin with mitochondrial physiology in each of these conditions, many of which have already been found to benefit from melatonin therapy. There are also nondiseased cells, often those that are rapidly proliferating, that utilize Warburg metabolism in lieu of OXPHOS. Since these are nondiseased cells, their mitochondria are presumably producing melatonin so the night-time blood melatonin rise performs its other essential function – that is, as a circadian-synchronizing agent.

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