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MINI-REVIEW

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Regulation of cancer cell glucose metabolism is determinant for cancer cell fate after melatonin administration

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Abstract

Several oncogenic pathways plus local microenvironmental conditions, such as hypoxia, converge on the regulation of cancer cells metabolism. The major metabolic alteration consists of a shift from oxidative phosphorylation as the major glucose consumer to aerobic glycolysis, although most of cancer cells utilize both pathways to a greater or lesser extent. Aerobic glycolysis, together with the directly related metabolic pathways such as the tricarboxylic acid cycle, the pentose phosphate pathway, or gluconeogenesis are currently considered as therapeutic targets in cancer research. Melatonin has been reported to present numerous antitumor effects, which result in a reduced cell growth. This is achieved with both low and high concentrations with no relevant side effects. Indeed, high concentrations of this indolamine reduce proliferation of cancer types resistant to low concentrations and induce cell death in some types of tumors. Previous work suggest that regulation of glucose metabolism and other related pathways play an important role in the antitumoral effects of high concentration of melatonin. In the present review, we analyze recent work on the regulation by such concentrations of this indolamine on aerobic glycolysis, gluconeogenesis, the tricarboxylic acid cycle and the pentose phosphate pathways of cancer cells.

KEYWORDS

aerobic glycolysis, gluconeogenesis, melatonin cytotoxicity, pentose phosphate pathway, TCA cycle, tumor metabolism

1 | INTRODUCTION

Melatonin is an indolamine produced by the pineal gland. This gland is responsible for the synthesis of plasma melatonin. However, most of the tissues are capable to synthesize this indolamine locally, highlighting its functional importance in maintaining cell biology and physiology. Plasma concentration is in the nanomolar range, whereas it presents concentrations several orders of magnitude higher in other fluids. Being the hormone responsible for the circadian rhythms, the study of its role and the therapeutic applications in this field continues to be a key point in its research today. However, it has been seen for decades that it presents many other therapeutic actions (Reiter, Rosales-Corral, Tan, Acuna-Castroviejo, et al., 2017). That could be summarized by saying that it behaves like a cytoprotective molecule against a wide range of insults in normal cells, in many of which its antioxidant properties are highly involved, while it presents antiproliferative and cytotoxic actions in tumor cells. Besides, no relevant side effects have been shown nor damage to normal cells in long-term in vivo experiments

Its antitumor effects occur at both, low (plasma levels) and high concentrations. In general, studies show that low concentrations decrease tumor growth, while high concentrations (a) increase efficacy in inhibiting cell growth in quite a few tumor cells; (b) make sensitive a lot of tumor types normally insensitive to low concentrations; and (c) induce cytotoxicity, mainly apoptosis, in some types of tumor cells (Rodríguez et al., 2013). The proapoptotic effects of melatonin on tumor cells began to be published about 15 years ago. It was remarkable from the first publications that these effects used to be associated with an increase in the early production of free radicals by tumor cells, different from the later one, associated with the apoptotic process itself. This turned out to be a bit counterintuitive, as the antioxidant properties of this indolamine were already well established in those years (Reiter, Rosales-Corral, Tan, Jou, et al., 2017; Rodríguez et al., 2004). The mechanisms used downstream of ROS increase to induce apoptosis after the administration of high melatonin concentrations were studied (Rodríguez et al., 2013). However, it remains a dilemma (a) how melatonin, an antioxidant molecule, induces apoptosis in some types of tumor cells by raising ROS and (b) why, contrary to what occurs in tumor growth inhibition—as occurs in almost all tumor cells sensitive to this indolamine-cytotoxicity only occurs in some tumor types. What do these particular tumor cells have so that melatonin induces cell death in them?

This review focuses on melatonin regulation of tumor glucose metabolism, one of the aspects of tumor biology that is currently part of a large number of studies aimed at finding new therapeutic targets for the design of new drugs against cancer. We start from the premise that melatonin, being a molecule with well-proven antioxidant effects, is not likely to increase cellular production of ROS because it has pro-oxidant effects per se. Taking into account publications reported in recent years, we propose that the increase in ROS in some types of tumor cells after treatment with high concentrations of melatonin is due to particular characteristics of said cells in relation to the type of glucose metabolism they present or with alterations in the mitochondria, being both likely associated.

1.1 | Aerobic glycolysis: The Warburg effect

Variations in the tumor microenvironment, such as hypoxia, and many intracellular pathways altered by oncogenic mutations converge in the regulation of tumor metabolism in response to the increased energy demand required for cell proliferation and survival. Cancer cells require an accelerated formation of ATP, an increased biosynthesis of macromolecules, and a finer regulation of the cellular redox state, where a slight increase in intracellular oxidants favors cell proliferation, while a significant increase can induce cell death.

One of the most important alterations in the regulation of tumor metabolism relates to glycolytic metabolism. These changes imply a shift from oxidative phosphorylation (OXPHOS) as the main energy source to the lactate synthesis pathway. In differentiated cells under normoxic conditions, the pyruvate obtained from glycolysis enters the mitochondria to join the tricarboxylic acid cycle (TCA cycle) and produce NADH. Electrons of NADH are used to obtain energy in the OXPHOS process, oxygen being the final electron acceptor. Under hypoxic conditions or when cells present a high proliferation rate, part of the pyruvate stops entering the mitochondria and is metabolized to lactate. This process is less efficient for obtaining energy from each molecule of glucose, but no oxygen is required. Also, proliferating tissues and tumor cells obtain a significant part of their energy through this second pathway even in the presence of oxygen. This process is known as aerobic glycolysis or Warburg effect (Warburg, 1956; Figure 1). It is important to highlight that the rate at which tumor cells use aerobic glycolysis as an energy source is not the same for all cancer types or in all cells within a given cancer. Although there are tumor cells in which there are mutations in the genes coding for the complexes of the electron transport chain, most complexes retain the capacity to use OXPHOS and can consume oxygen as normal cells do. Even tumors with marked glycolytic metabolism do not totally abandon OXPHOS (Martinez-Outschoorn, Lisanti, & Sotgia, 2014).

Using aerobic glycolysis, cells get less energy from each molecule of glucose (Cox & Bonner, 2001), but this is compensated by two main advantages of this process. First, aerobic glycolysis is faster and takes place a greater number of times (Pfeiffer, Schuster, & Bonhoeffer, 2001). Glucose uptake increases, as long as it is available in the extracellular media of the tumor microenvironment, and more energy is obtained per time unit than would be through the TCA cycle. And second, some of the subproducts deviate at different points of the pathway toward the synthesis of lipids, proteins, and nucleic acids, all of which are abundantly needed by proliferating tumor cells (DeBerardinis, Lum, Hatzivassiliou, & Thompson, 2008). Clinical data confirmed that the deviation from OXPHOS to aerobic glycolysis favors resistance to chemotherapy, while return to OXPHOS turns cancer cells sensitive to chemotherapy again (Zhao, Butler, & Tan, 2013).

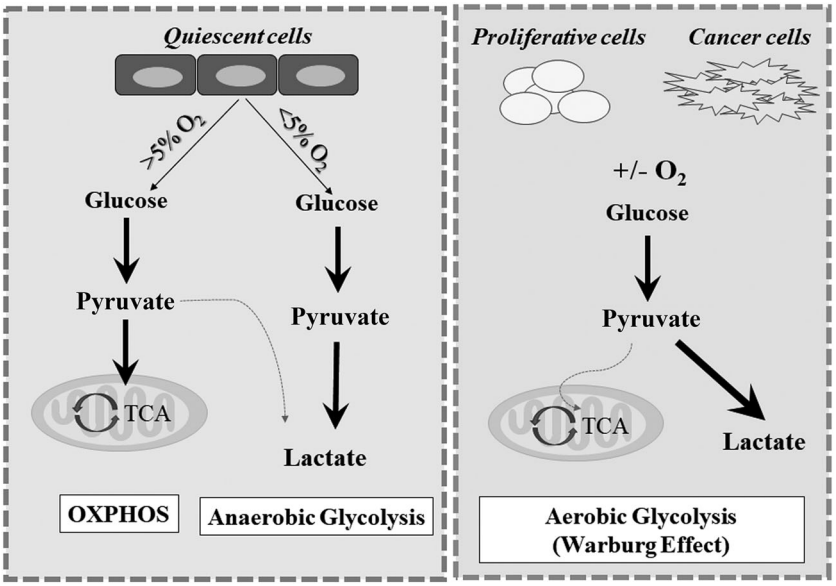

FIGURE 1 Glucose metabolism in proliferative and cancer cells versus quiescent cells

The change of metabolism from OXPHOS to aerobic glycolysis is driven by the hypoxia inducible transcription factor (HIF-1) or by mutations in several oncogenes and tumor suppressors, such as the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway, p53, or MYC (Cairns, Harris, & Mak, 2011). Considering that inhibition of aerobic glycolysis decreases resistance to chemotherapeutic drugs, HIF-1 became a major therapeutic target for which inhibitory compounds are currently being developed (Masoud & Li, 2015). HIF-1 is a heterodimeric transcription factor composed of two subunits, HIF-1α and HIF-1β (G. L. Wang, Jiang, Rue, & Semenza, 1995). HIF-1α is an oxygen-sensitive subunit and is normally hydroxylated by the action of a group of enzymes called prolyl-4-hydroxylases in an oxygen-dependent manner. Hydroxylation promotes its association with the tumor suppressor von Hippel-Lindau (VHL), marking it for ubiquitination and later degradation. When hypoxia occurs, hydroxylation of HIF-1α is inhibited, resulting in its stabilization, translocation to the nucleus, and binding to the HIF-1β subunit (Figure 2; Masoud & Li, 2015). This heterodimeric complex then increases the transcription of genes involved in glucose transport and glycolysis, among others (Semenza, 2010).

In addition to hypoxia, oncogenic signals also have a function in HIF-1α regulation in cancer cells. HIF-1α is constitutively transcribed, synthesized, and stabilized through a series of signaling events involving several growth factors and other signaling molecules. Among

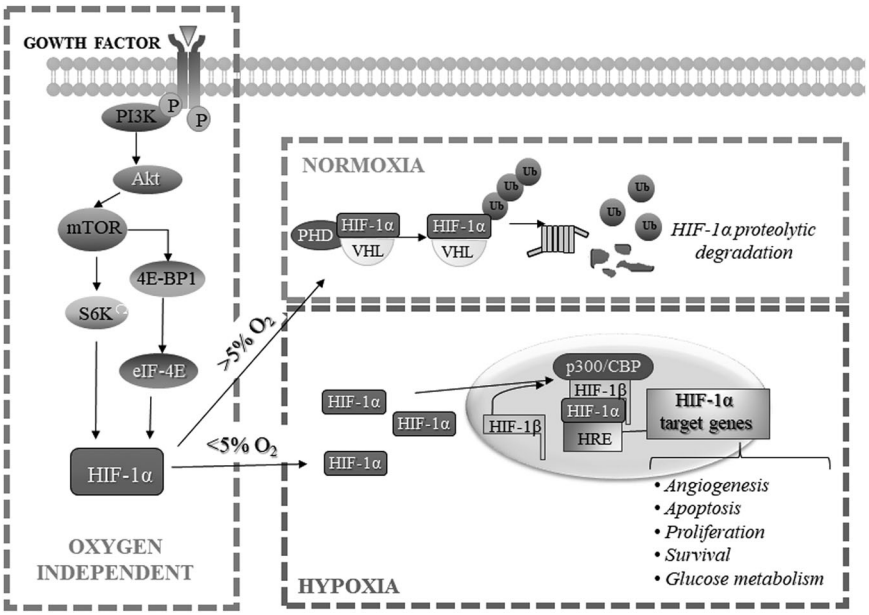

FIGURE 2 Hhypoxia inducible transcription factor-1α (HIF-1α) regulation by oncogenic signaling (left side) and by hypoxia (right side)

these are the signaling pathway PI3K/Akt/mTOR (Majumder et al., 2004), VHL mutations (Ivan & Kaelin, 2001), mutations in the enzymes succinate dehydrogenase (SDH) and fumarate hydratase (FH; Isaacs et al., 2005; Selak et al., 2005), or an increase in reactive oxygen species (ROS; Zheng et al., 2014).

Together with HIF-1α, the PI3K/Akt/mTOR pathway is considered a master regulator of aerobic glycolysis. Constitutive activation of Akt is one of the most important alterations in cancer also in terms of metabolism, as it is sufficient to induce aerobic glycolysis and lactate production (Elstrom et al., 2004). Akt increases gene expression of glucose transporters; increases the phosphorylation of key enzymes in glycolysis, such as hexokinase and phosphofructokinase 2; inhibits FOXO transcription factors, which will result in changes in gene expression that favor aerobic glycolysis; and activates mTOR, which promotes the translation of messenger mRNA and synthesis of macromolecules, including Hif-1α (Cairns et al., 2011; Guertin & Sabatini, 2007). Finally, other oncogenes and tumor suppressor genes may also regulate tumor metabolism and particularly aerobic glycolysis. The MYC oncogene has been reported to increase glucose transporters and glycolysis enzymes, lactate dehydrogenase (LDH, the enzyme responsible for the conversion of pyruvate to lactate) and cytoplasmic pyruvate dehydrogenase kinase (PDK; Figure 3; Kim, Gao, Liu, Semenza, & Dang, 2007). Finally, mutation of the p53 tumor suppressor causes, among other effects, an increase in the expression of the hexokinase 2 (HK2), the enzyme that catalyzes the first step in glycolysis (Vousden & Ryan, 2009).

Thus, aerobic glycolysis has become in recent years an important factor in cancer research. It is considered now as a hallmark of cancer and the study of new drugs able to inhibit tumor metabolism is a rapidly developing field of research (Kishton & Rathmell, 2015).

1.2 | Gluconeogenesis

Gluconeogenesis is the metabolic pathway that allows the synthesis of glucose from nonglucidic precursors (pyruvate, lactate, glycerol, intermediates of the TCA cycle, and some amino acids). Although gluconeogenesis uses mostly the same enzymes as glycolysis (most are reversible reactions), there are three enzymes specific to this route (Figure 3). These are (a) phosphoenolpyruvate carboxykinase (PCK, encoded by the PEPCK gene)—which catalyzes the conversion of oxaloacetate to phosphoenolpyruvate (PEP)—the first and rate-limiting step in gluconeogenesis; (b) fructose-1,6-biphosphatase (FBP1), which converts fructose-1,6-bisphosphate (F1,6P) into fructose-6-phosphate (F6P); and (c) glucose-6-phosphatase (G6Pase), which catalyzes the conversion of glucose-6-phosphate (G6P) into glucose and orthophosphate.

PCK can be located in the cytoplasm (PCK1, PEPCK-C) or in the mitochondria (PCK2, PEPCK-M). PCK1 is relatively specific of glycogenic tissues (liver, kidney, small intestine, and adipose tissue), as well as their tumor counterparts; its overexpression, however, has recently been demonstrated in other tumor types such as colon cancer (Montal et al., 2015). Conversely, PCK2 is more widespread among normal tissues (pancreas, lymphocytes, or neurons), as well as tumors (breast, acute lymphoid leukemia, glioblastoma, neuroblastoma, and osteosarcoma; Stark & Kibbey, 2014). Due to its key role in liver glycogenesis, the most studied enzyme is PCK1, but the fact that PCK2 is present in most tissues suggests that it has an important and defined role in cellular metabolism. When there is an excess of acetyl-CoA in the TCA cycle, or there is a shortage of nutrients (glucose, amino acids), the pyruvate carboxylase (PC) is activated in the mitochondria, converting pyruvate to oxaloacetate. Oxaloacetate is then converted to PEP by PCK2, and transported out of the mitochondria. As PEP is a glycolysis–gluconeogenesis intermediate, PCK2 could also be a link between TCA and gluconeogenesis, favoring metabolic pathways starting from this route, such as the pentose phosphate, serine, glycerol, or glycogen synthesis pathways (Méndez-Lucas, Hyrossova, Novellasdemunt, Viñals, & Perales, 2014).

Apart from the change from oxidative metabolism to fermentative metabolism (aerobic glycolysis), which occurs to a greater or lesser degree in many tumor cells, activation of gluconeogenesis has also been demonstrated in some of them (Leithner, Hrzenjak, & Olschewski, 2014). Gluconeogenesis is a metabolic pathway that runs in the opposite direction to glycolysis, forcing pyruvate to convert to glucose or intermediate metabolites that then lead to other biosynthetic pathways. Thus, the primary role of gluconeogenesis in tumor cells is to provide intermediate metabolites for the synthesis of biomolecules, something essential for the growth of tumor cells, especially when nutrients are scarce in the tumor microenvironment (Zhang et al., 2014).

Under conditions of nutrient deficiency, much tumors increase the expression of PCK2, which behaves as a survival molecule (Méndez-Lucas et al., 2014; Vincent et al., 2015). However, it seems that in other tumor types this pathway is downregulated instead, also as a survival mechanism. Thus, the cytosolic variant of this enzyme, PCK1, is diminished in hepatocellular or kidney carcinomas, where metabolic reprogramming by activation of this enzyme kills tumor cells (Liu et al., 2018; R. Ma et al., 2013). Luo et al. (2017) demonstrated that downregulation of PCK2 in tumor-repopulating cells (TRCs) of melanoma increases the transport of TCA intermediates from the mitochondria to the cytosol, pushing acetyl-CoA toward the synthesis of fatty acids. When oxaloacetate does not convert to PEP, citrate exits the mitochondria and it is deviated toward the formation of fatty acids. These events decrease the flow of carbons toward the production of malate, accumulating fumarate in the TCA, which, in turn, induces stabilization of the HIF-1α transcription factor. Additionally, TRCs showed higher glucose consumption and a higher proliferation rate. Consistently, overexpression of this enzyme reduces glucose consumption, tumor growth, citrate exit from the mitochondria, fumarate levels, and HIF-1α stabilization in TRCs of melanoma. Finally, TRCs have limited overall O_2 consumption and PCK2 overexpression increases it, indicating that PCK2 also regulates, directly or indirectly, OXPHOS. PCK2 overexpression not only decreases the growth of TRCs in the culture but also abolishes their tumorigenicity in vivo. Consistent with this, Park et al. (2014)

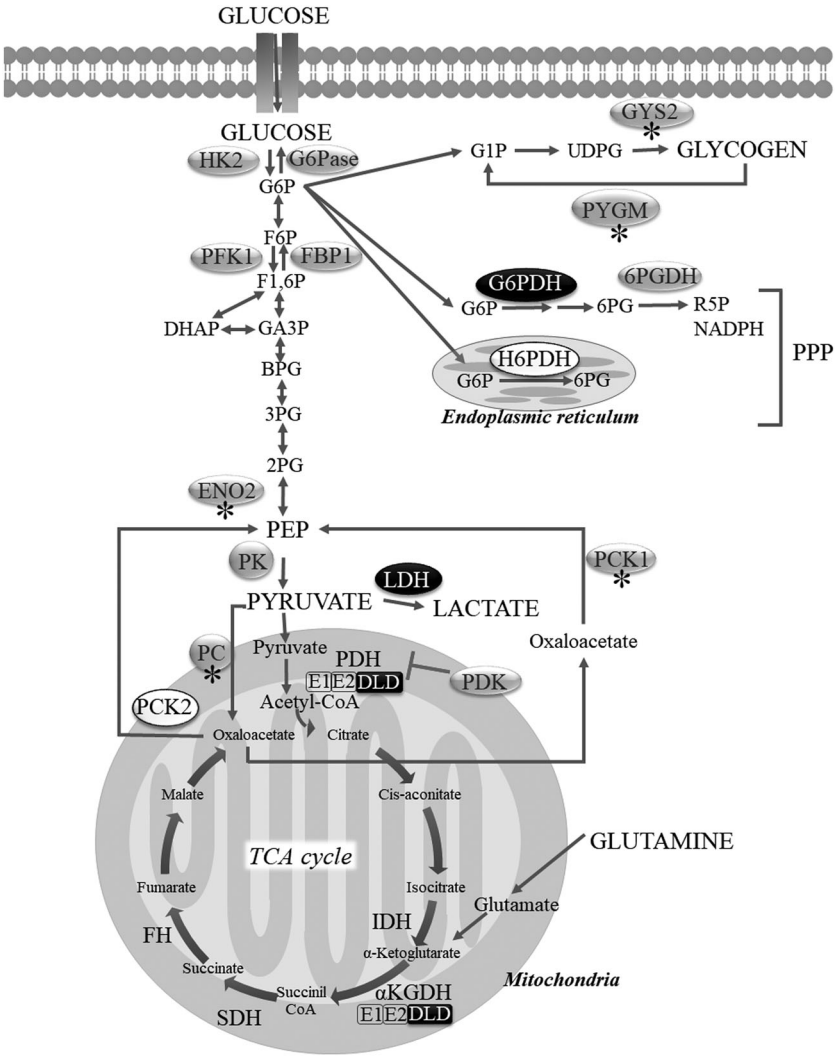

FIGURE 3 Glycolysis and its coupling to tricarboxylic acid cycle (TCA), gluconeogenesis, pentose phosphate pathway, and glycogen metabolism are represented. Melatonin effects on enzymes involved in glycolysis and its coupled metabolic pathways TCA, gluconeogenesis and pentose phosphate pathway and glycogen metabolism are highlighted. Enzymes in white are overexpressed; enzymes in black are suppressed (in the case of LDH and G6PDH, decreased activity is represented); enzymes with asterisk are overexpressed only in one of the FLT3-ITD LMA cell lines reported. Abbreviations: (1) Glycolysis and related pathways of metabolic intermediates: G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; F1, 6P, fructose-1, 6-biphosphate; DHAP, dihydroxyacetone phosphate; GA3P, glyceraldehyde-3-phosphate; BPG, biphosphoglycerate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate. 6PG, 6-phosphogluconate; R5P, ribose-5-phosphate; G1P, glucose-1-phosphate; UDPG, uridine diphosphate glucose. (2) Some of the glycolytic and related metabolic pathways enzymes: HK2, hexoquinase 2; G6Pase, glucose-6 phosphatase; PFK1, phosphofructokinase; FBP1, fructobiphosphatase 1; ENO2, enolase; PK, pyruvate kinase; LDH, lactate dehydrogenase; PC, pyruvate carboxylase; PCK2, phosphoenolpyruvate carboxykinase 2 (mitochondrial PCK); PCK1, phosphoenolpyruvate carboxykinase 1 (cytoplasmic PCK); PDH, pyruvate dehydrogenase; E1, enzyme 1 (of the complexes PDH and αKGDH); E2, enzyme 2 (of the complexes PDH and αKGDH); DLD, dihydrolipoamide; PDK, pyruvate dehydrogenase kinase; IDH, isocitrate dehydrogenase; αKGDH, α-ketoglutarate dehydrogenase; SDH, succinate dehydrogenase; FH, fumarate hydratase; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; H6PDH, hexose-6-phosphate dehydrogenase; GYS, glycogen synthase; PYGM, glycogen phosphorylase (muscle)

observed that the resistance of colon cancer cells to 5-FU was inversely correlated to PCK2 expression levels, and that further suppression of PCK2 expression decreased both PEP levels and the susceptibility of TRCs to 5-FU.

In summary, in most tumoral cells PCK2 activity is increased, activating gluconeogenesis and deviating the intermediate metabolites from the TCA cycle toward other synthetic routes, such as the pentose phosphate pathway (PPP). On the contrary, when PCK2 activity is diminished, in certain types of tumor cells, TCA intermediates such as fumarate accumulate, stabilizing HIF-1α, the master regulator of glycolysis. Whether PCK2 is activated or inhibited, it plays a vital role in tumor survival: its role depends on the type of tumor cells and the microenvironmental context where they develop.

1.3 | The TCA cycle

The TCA cycle is a key pathway for the production of energy, the synthesis of macromolecules, and the control of the cellular oxidative state. It involves the incorporation of molecules that come from the catabolism of glucose, fatty acids, and proteins (anaplerosis) to produce ATP, NADH (reducing power), and other molecules that leave the cycle to be incorporated into the synthetic pathways of macromolecules, such as fatty acids and cholesterol (cataplerosis).

The main molecules that enter the TCA cycle are acetyl-CoA, from glycolysis and the oxidation of fatty acids, and α-ketoglutarate, from the catabolism of amino acids (glutamine; Figure 3). Additionally, other intermediate metabolites from the catabolism of fatty acids and other amino acids can be incorporated into the cycle (DeBerardinis et al., 2007). The main molecules that leave the TCA cycle are citrate (for biosynthesis of fatty acids and cholesterol) and oxaloacetate (toward gluconeogenesis). Additionally, malate and other metabolites can also escape. TCA cycle enzymes and the transporters of the different molecules entering or leaving the cycle are regulated by various oncogenes and tumor suppressors (Chen & Russo, 2012).

Various TCA enzymes are mutated in several types of cancer. As an example, mutations in SDH gene and reduced expression of this enzyme, that takes part in both the TCA cycle and the mitochondrial electron transport chain (Complex II), have been reported in several types of cancer (Hao et al., 2009; Ricketts et al., 2012). Defects in both SDH and FH with the consequent accumulation of succinate and fumarate are currently known to regulate metabolic changes in cancer cells toward aerobic glycolysis by promoting HIF-1α stabilization (King, Selak, & Gottlieb, 2006), while α-ketoglutarate accumulation is known to promote HIF-1α degradation (Tennant et al., 2009).

Although the TCA cycle is used by all cells, cancer cells seem to be more sensitive to its inhibition (Kishton et al., 2016) and several of its enzymes may serve as targets for the design of new therapies. In this sense, an α-ketoglutarate dehydrogenase (α-KGDH) inhibitor is undergoing a phase II clinical trial with high tolerance on the part of patients (Lycan et al., 2016).

1.4 | The PPP

Some of the intermediates of glycolysis and gluconeogenesis are directed to the well-known PPP. This route is used to obtain ribose, necessary for the synthesis of nucleotides and nucleic acids, and nicotinamide adenine dinucleotide phosphate (NADPH). NADPH serves as fuel for the synthesis of macromolecules, such as lipids, but also has a reducing potential that can counteract the reactive oxygen species formed during cell proliferation. The PPP has two phases: oxidative and nonoxidative. In the first phase, the oxidation of glucose-6-phosphate (G6P), an intermediate metabolite in glycolysis and gluconeogenesis, produces ribulose-5-phosphate (Figure 3). Glucose-6-phosphate dehydrogenase (G6PDH) is the first and rate-limiting enzyme in the PPP, and its inhibition has been shown to be lethal for certain cancer cells (Cho, Cha, Kim, Kim, & Yook, 2018). A phosphogluconolactone and a molecule of NADPH are formed in the reaction catalyzed by G6PDH. By means of the phosphogluconolactonase enzyme, phosphogluconolactone becomes 6-phosphogluconate (6PG). Finally, the 6-phosphogluconate dehydrogenase (6PGDH) will catalyze the conversion of 6PG into ribulose-5-phosphate and other molecule of NADPH. The enzyme pentose-5-phosphate isomerase isomerizes ribulose-5-phosphate (R5P), converting it into ribose-5-phosphate (RI5P) and xylulose-5-phosphate (X5P). The second, nonoxidative phase is basically a series of reversible reactions, which are redirected in accordance with cell necessities. When the cells need antioxidant molecules that depend on NADPH, that is glutathione, the nonoxidative phase goes from RI5P and X5P toward glyceraldehyde-3-phosphate (GA3P) and fructose-6-phosphate (F6P), both intermediate metabolites of glycolysis. The former continues downstream glycolysis while the latter follows upstream glycolysis to replenish G6P levels and to start again the oxidative phase of PPP. When highly proliferative cells (tumor cells included) need nucleic acids, this nonoxidative phase of the PPP supplies pentose sugars for DNA and RNA synthesis directly from GA3P and F6P (for a detailed review of PPP, see Patra & Hay, 2014; Riganti, Gazzano, Polimeni, Aldieri, & Ghigo, 2012).

Therefore, PPP is intimately connected to glycolysis and gluconeogenesis. Cancer cells regulate the glycolysis-control enzymes (that catalyze nonreversible reactions), phosphofructokinase 1 (PFK1) and pyruvate kinase (PK), according to the microenvironmental circumstances and cellular necessities to increase glycolysis flux toward the PPP. As an example, hypoxia and cyclin D3/CDK6 complex inhibit PFK1 (H. Wang et al., 2017), and ROS and CDK6 inhibit PK in cancer cells (Anastasiou et al., 2011), thereby decreasing glycolysis and redirecting carbon flux to the PPP. In addition, G6PDH, the gatekeeper of the PPP, is regulated by several oncogenes, with p53 being one of the best studied. P53 inhibits G6PDH, but its mutated forms lack this activity, thereby increasing PPP activity, NADPH formation, and glucose consumption (Jiang et al., 2011).

Liu et al. (2018) have found that in tumor cells where PCK1 is not elevated compared with normal cells of similar tissue, PEP increases because of the activation of the gluconeogenesis pathway when this enzyme is overexpressed, as expected. Then, as a consequence of the reversible reactions of the enzymes enolase (ENO2) and phosphoglycerate mutase, there is also a rise in 3-phosphoglycerate (3PG). These authors suggest a relationship between the increase in gluconeogenesis and inhibition of PPP, as 3PG inhibits the activity of 6PGDH (Hitosugi et al., 2012). They verified that RI5P, the last product of the oxidative phase of the PPP and essential for the synthesis of nucleotides and nucleic acids, was reduced after overexpression of PCK1. The ineffectiveness of the oxidative phase of the PPP after this inhibition also made the cells less protected against oxidative stress, reporting elevated levels of oxidative stress and a reduction in GSH/GSSG ratios. This could be (besides the destabilization of HIF-1α due to decrease of accumulation of fumarate)

another reason why overexpression of PCK inhibits cell growth in the tumors where these enzymes are not elevated.

Besides the cytoplasmic PPP, a similar pathway has been reported recently in the endoplasmic reticulum, where the first and rate-limiting enzyme is hexose-6-phosphate dehydrogenase (H6PDH; Marini et al., 2016). This enzyme has been classically related to signaling pathways, linking activation of steroids mediated by NADPH (Bánhegyi, Benedetti, Fulceri, & Senesi, 2004). However, a new function reported by Marini et al. (2016) relates H6PDH to the consumption of glucose derivatives in cancer, and clearly deserves further research.

2 | IMPLICATIONS OF THE REGULATION OF GLYCOLYTIC METABOLISM AND RELATED METABOLIC PATHWAYS FOR THE CYTOTOXIC EFFECT OF MELATONIN

Although a multitude of genetic and epigenetic differences between tumors has been described, therapies directed against altered signaling pathways, particularly in certain tumor types, have not achieved the expected success. In addition, another feature of tumor biology, the study of which is receiving great interest and the findings of which are the basis for the design of new therapeutic strategies, is the change in glucose metabolism displayed by virtually all tumors (Gatenby & Gillies, 2004). As mentioned before, these alterations attempt to maintain a balance to sustain the increase in proliferation by procuring biomolecules for the new cells and providing energy without fatally altering the cellular redox state.

2.1 | Relationship between melatonin, mitochondria, tumor metabolism, and increased ROS

Melatonin induces cytotoxicity at high concentrations (μM–mM) by increasing the level of reactive oxygen species (ROS) in certain tumor cell lines in culture (Bejarano et al., 2010; Buyukavci et al., 2006; Casado-Zapico et al., 2011; García-Santos et al., 2012; Prieto-Domínguez et al., 2016), although its antioxidant capacity is widely demonstrated in other biological contexts (Reiter, Rosales-Corral, Tan, Jou, et al., 2017; Rodríguez et al., 2004). Both the cytotoxicity of melatonin and the rise in ROS are counteracted by the administration of antioxidants, which supports the involvement of these toxic oxygen derivatives in the cytotoxicity of this molecule in certain tumor types (Sánchez-Sánchez et al., 2011). The main question remains is why melatonin, an antioxidant, increases ROS in some types of tumor cells? Most ROS are generated in the mitochondria during electron transport throughout OXPHOS. The increase in ROS in tumor cells is counteracted by endogenous antioxidant molecules, which in tumors are also produced in high amounts. NADPH (the reducing molecule needed for glutathione and thioredoxin to work as antioxidants) and NADH produced during several metabolic processes, are among the most important, with many originating from PPP and TCA cycle, respectively. If ROS are produced in greater amount or if the reducing capacity of the cell is diminished, the redox balance is disturbed and the additional increase produces toxicity and cell death. Thus, melatonin increases ROS in specific tumor types either because (a) it increases its production or (b) it reduces the generation of reducing molecules such as NADPH/NADH by altering tumor metabolism. Perhaps both of these processes are involved.

The first option could occur, for example, if melatonin activates altered enzymes in the electron transport chain. Certain tumors present mutations in several of these enzymes (Brandon, Baldi, & Wallace, 2006) and as a result the mitochondria may contribute to metabolic alterations in cancer (Frezza & Gottlieb, 2009). Melatonin is able to activate several electron transport chain complexes, especially complex I, in normal cells (Martín et al., 2000). Thus, if melatonin activates mitochondrial complexes also in cancer cells in which they are altered, there could induce a loss of electrons that would explain the observed increase in ROS. Consistently, whenever P19 tumor stem cells are forced to use OXPHOS by decreasing glucose in the culture medium, and then treated with melatonin, there is an increase in ROS production, a decrease in oxygen consumption, and cytotoxicity (Loureiro et al., 2015). It remains to be shown, however, whether they have any of the electron transport complexes actually altered. Also, Shen et al. (2018) have reported, in head and neck cancer cells, that melatonin increases mitochondrial complexes I, III, and IV and the mass of nonfunctioning mitochondria, as well as induces cell death. This would support mitochondrial alteration as a possible source of ROS increase and cell death after melatonin administration in some types of cancer cells.

A decrease in the reducing potential may take place if melatonin limits NADH/NADPH formation by altering tumor metabolism. Among the changes that characterize tumor metabolic activity, the increase in the production of ROS and at the same time, the rise in the production of molecules with reducing power is substantial. These molecules, primarily NADPH and NADH, are produced mainly through glycolysis, PPP, and TCA cycle. If melatonin alters any of these processes, the cellular redox balance might be shifted toward the accumulation of oxidant molecules in the cell.

2.2 | Melatonin regulation of aerobic glycolysis

It has recently been shown that high concentrations of melatonin alter tumor metabolism by inhibiting aerobic glycolysis (Warburg effect) in cells in which it induces ROS increase and cytotoxicity (Ewing's sarcoma cells), whereas it does not induce such alterations in tumor cells where it only inhibits cell proliferation (chondrosarcoma cells; Sanchez-Sanchez et al., 2015). Moreover, the cells where melatonin promotes cytotoxicity depend to a large extent on aerobic glycolysis for survival (Sánchez-Sánchez et al., 2011). In such cells, administration of melatonin results in: (a) increase of destabilized HIF-1α, the master regulator of aerobic glycolysis; (b) a drop in glucose uptake; (c) a rapid loackss of glycogen stores, in an attempt

to maintain the energy sources of the cell; (d) the inhibition of LDH; and (e) a decrease in lactate production. Warburg effect indicators do not change in tumor cells where melatonin inhibits proliferation without inducing cell death.

Recent studies describing inhibition of aerobic glycolysis by elevated concentrations of melatonin have been also reported in prostate cancer cells (Hevia et al., 2017) and acute myeloid leukemia (AML) with an internal tandem duplication (ITD) in the FMS-like tyrosine kinase 3 (FLT3) receptor gene—a mutation that results in a poor prognosis in these patients (Puente-Moncada et al., 2020). FLT3-ITD signaling is involved in the increase of aerobic glycolysis (Ju et al., 2017), while its inhibition has been reported to decrease Warburg effect and induce cell death (Puente-Moncada et al., 2018). It was demonstrated that melatonin also increases destabilized HIF-1α, inhibits aerobic glycolysis, and induces cytotoxicity in the FLT3-ITD cells, MV-411 and MOLT13, but not in AML cells lacking the FLT3-ITD mutation (Puente-Moncada et al., 2020). Also, Sonehara et al. (2019), using an experimental model mimicking the acidification of the microenvironment produced during aerobic glycolysis, found that melatonin decreases the expression of the glucose transporter GLUT-1, decreases cell proliferation, and induces apoptosis in breast cancer cell lines.

Studies on the regulation of aerobic glycolysis-related proteins by melatonin have also been performed in an experimental model of ovarian cancer in vivo (Chuffa et al., 2016). Although the study is not comparable in terms of concentration of melatonin administered, as the concentration of this molecule in the blood was 70 ng/ml, the increase in this concentration with respect to untreated animals was doubled. It must also be noted that blood concentrations in vivo are not similar to those used in cell cultures, as the in vivo concentrations in different fluids, organs or tissues, or in the extracellular microenvironment of cancer cells is not known (Rodríguez et al., 2013). In the Chuffa et al. (2016) study, tumor mass and volume decreased by 40%, although it was not determined whether this reduction was the result of decreased proliferation or of cell death. Even taking into account that the conditions of the tumor microenvironment in vivo are different from those found in cell cultures, the study showed that melatonin induced a drop in several enzymes involved in the signaling pathway of HIF-1α and in aerobic glycolysis, highlighting LDH-A or pyruvate kinase (A and B) among others. Antioxidant enzymes such as Cu–Zn superoxide dismutase (Cu–Zn SOD) or thioredoxin were also diminished.

Likewise, an in vivo experimental model of leiomyosarcoma, Mao et al. (2016) observed an inhibition of aerobic glycolysis by melatonin. They also found a drop in lactate levels and glucose uptake, two of the key features of aerobic glycolysis.

Although not much research exists yet related to Warburg effect as influenced by melatonin, HIF-1α has been reported to be less expressed or destabilized by high concentrations of melatonin in several types of cancer cell lines in culture (in addition to those mentioned above) such as prostate cancer cells (Park, Hwang, Suh, & Baek, 2009), hepatocellular carcinoma (Colombo, Wolf Maciel, Carvalho Ferreira, & Ferreira Da Silva, 2016; Prieto-Domínguez et al., 2017), oral cancer cells (Goncalves et al., 2014), or lung cancer cells (Lee, Lee, Moon, & Park, 2014). These cancer cells lines have been reported to be sensitive to the cytotoxic actions of high concentrations of melatonin (Joo & Yoo, 2009; Z. Ma et al., 2019; Martín-Renedo et al., 2008; Ordoñez et al., 2015).

The increase of destabilized HIF-1α after the administration of high concentrations of melatonin is a fact shared by all studied cancer cells in which this indolamine regulates aerobic glycolysis (Puente-Moncada et al., 2020; Sanchez-Sanchez et al., 2015, 2011). However, there are no studies that demonstrate the mechanisms involved in such a change.

As we discussed in Section 1, the PI3K/Akt/mTOR pathway is, along with hypoxia, the main regulator of HIF-1α. Inhibition of Akt by melatonin in tumor cells has been previously reported in other types of cancer (Martín et al., 2006, 2010). Puente-Moncada et al. (2020) also reported the inhibition by melatonin of the PI3K/Akt/mTOR pathway, which could explain the inhibition of HIF-1α (Majmundar, Wong, & Simon, 2010). However, not all cancer cells in which melatonin regulates tumor metabolism and induces cell death show a decrease in phosphorylated Akt after its administration (Sanchez-Sanchez et al., 2015), so other mechanisms should be studied. For a summary of some of the pathways regulated by melatonin that could be involved in decrease of active HIF-1α and cell death, see Figure 4.

One of the effects of melatonin that could also explain the decrease in active HIF-1α after its administration is the mitochondrial dysfunction shown in tumor cells (Shen et al., 2018), as it has been shown that it can repress the synthesis of HIF-1α protein in hepatocarcinoma cells (Hsu et al., 2013). These latter authors propose that HIF-1α decrease can be mediated by activation of the cellular energy sensor 5′-adenosine monophosphate kinase (AMPK), which is also involved in downregulation of HIF-1α (J. C. Wang et al., 2018) and it is activated by melatonin in cancer cells (Mi et al., 2018). The tumor suppressor protein p53, in addition to inducing apoptosis after DNA damage and other cellular insults, thus participating in cancer prevention, has been shown to inhibit HIF-1α, the main regulator of aerobic glycolysis, in tumor cells (Ravi et al., 2000). Melatonin increases p53 expression in different cancer cell lines (Gelaleti et al., 2017; Martín-Renedo et al., 2008), so the implication of this pathway in the inhibition of aerobic glycolysis with cytotoxicity due to high concentrations of this indolamine should also be explored. Finally, α-ketoglutarate shows opposite effects to succinate and fumarate in regulating HIF-1α. α-Ketoglutarate promotes the hydroxylation of HIF-1α by reactivating prolyl hydroxylases (Tennant et al., 2009). The decrease in DLD expression after treatment with melatonin (Puente-Moncada et al., 2020), in addition to slowing down the TCA cycle, could cause an increase in α-ketoglutarate that could activate prolyl hydroxylases and therefore the hydroxylation of HIF-1α.

Melatonin also induces alterations of several genes involved in glucose metabolism in the AML cell lines with the FLT3-ITD mutation (Puente-Moncada et al., 2020). For alterations of metabolic key points and enzymes by melatonin, see Figure 3. There are two genes that are overexpressed in the two FLT3-ITD cell lines upon melatonin treatment: PEPCK-M (coding for the PCK2 enzyme) and H6PD

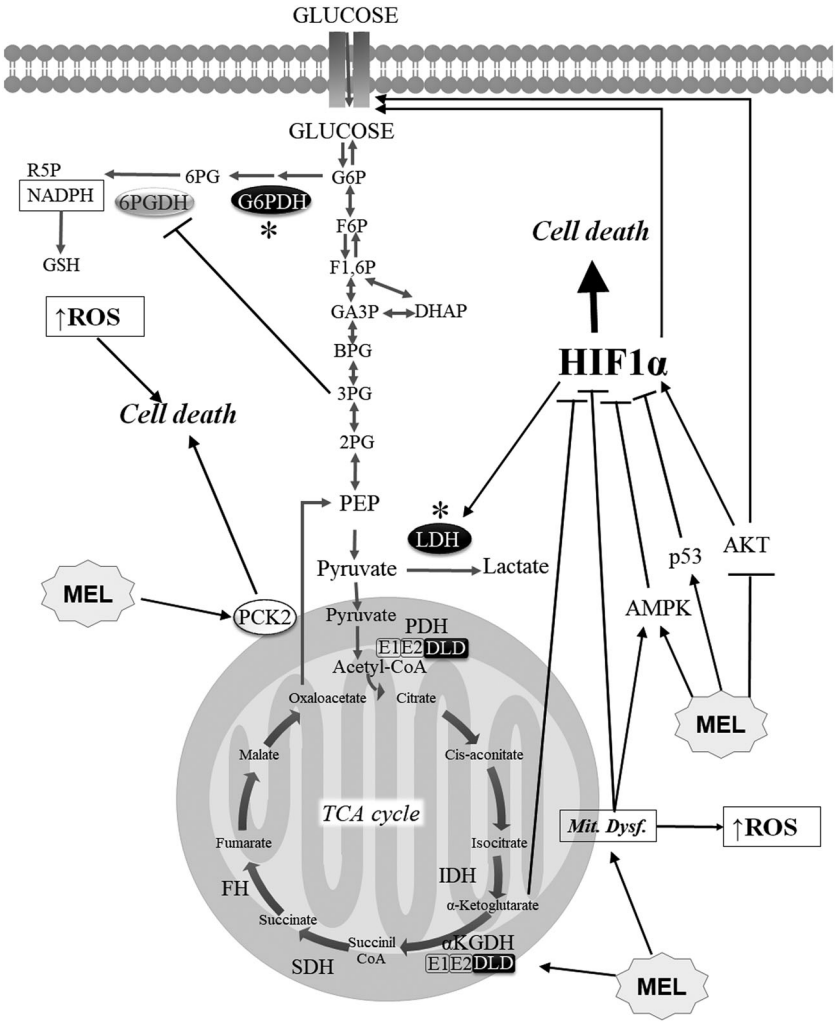

FIGURE 4 Proposed hypothesis to explain the induction of tumor cell death by melatonin (MEL) through its regulation of tumor metabolism and its effects on the mitochondria. (A) MEL inhibits HIF-1α, which reduces aerobic glycolysis and induces cell death. This inhibition could be due to: (1) accumulation of α-ketoglutarate in the TCA cycle due to decreased expression of dihydrolipoamide (DLD); (2) a possible induction of mitochondrial dysfunction (*Mit. Dysf.*); (3) Akt inhibition (not demonstrated in all cases); (4) a possible increase in p53; and 5) possible activation of the energy sensor AMP kinase (AMPK). (B) MEL increases the expression of PCK2 (which in some tumor types would induce cell death), increasing gluconeogenesis. (C) MEL increases reactive oxygen species (ROS), which cause cell death. This increase may be due to: (1) Possible induction by MEL of mitochondrial dysfunction; (2) Decrease in antioxidant molecules such as reduced glutathione (GSH) due to the low production of NADPH by the pentose phosphate pathway. Decrease on NADPH production by the PPP may be caused by the inhibition of glucose-6-phosphate dehydrogenase (G6PDH) activity. Also 6-phosphogluconate dehydrogenase (6PGDH) may be inhibited after the increase of gluconeogenesis and its intermediate metabolite 3-phosphoglycerate (3PG). Asterisk: enzymes with decreased activity. Enzymes in black are suppressed (in the case of DLD, decreased expression is represented). Enzymes in white are overexpressed. Other abbreviations: (1) Glycolysis metabolic intermediates: G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; F1, 6P, fructose-1, 6-biphosphate; DHAP, Dihydroxyacetone phosphate; GA3P, glyceraldehyde-3-phosphate; BPG, biphosphoglycerate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate. (2) Tricarboxylic acid cycle (TCA cycle) enzymes: PDH, pyruvate dehydrogenase; E1, enzyme 1 (of the complexes PDH and αKGDH); E2, enzyme 2 (of the complexes PDH and αKGDH); DLD, dihydrolipoamide; IDH, isocitrate dehydrogenase; αKGDH, α-ketoglutarate dehydrogenase; SDH, succinate dehydrogenase; FH, fumarate hydratase. (3) Gluconeogenesis: PCK2, phosphoenolpyruvate carboxykinase 2. (4) Pentose phosphate pathway: 6PG, 6-phosphogluconate; R5P, ribose-5-phosphate

(coding for H6PDH). Another gene is downregulated, namely dihydrolipoamide dehydrogenase (DLD), an enzyme that is part of the pyruvate dehydrogenase (PDH) and α-KGDH enzymatic complexes (both being part of the TCA cycle).

Hence, gene expression regulation by melatonin could directly alter both TCA cycle and gluconeogenesis.

2.3 | Melatonin regulation of the TCA cycle in cancer cells

Inhibition of DLD by melatonin (Puente-Moncada et al., 2020) has two possible major consequences. First, it can inhibit the conversion of pyruvate to acetyl-CoA and therefore the incorporation of carbon

atoms resulting from glycolysis into the TCA cycle. Second, it can inhibit the progression of the TCA cycle from α-ketoglutarate, the intermediate metabolite coming from the TCA cycle itself (from the isocitrate) and from the metabolism of glutamine. Therefore, melatonin can inhibit the TCA cycle by preventing the utilization of carbon atoms coming from glucose and glutamine, and eventually induce an accumulation of unused α-ketoglutarate. These data probably explain the drop in several intermediate metabolites of the TCA cycle observed after treatment with melatonin (Hevia et al., 2017). Decreased levels of α-ketoglutarate activate HIF-1α (Majmundar et al., 2010), and α-ketoglutarate accumulation activates prolyl hydroxylases increasing HIF-1α hydroxylation and deactivation (Tennant et al., 2009). Melatonin's inhibition of DLD (α-KGDH enzymatic complex) probably accounts for the increase of destabilized HIF-1α by the indolamine in the FLT3-ITD LMA cells (Puente-Moncada et al., 2020; Figure 4). Conversely, the decrease of α-KGDH complex would result in a reduction in succinate (that in turn inhibits HIF-1α hydroxylation when it accumulates); this may also contribute to the decrease of active HIF-1α.

The other major source of anaplerosis for TCA is glutamine. Although there are no studies on the effect of melatonin on the metabolism of this amino acid in tumor cells, it has been shown that this indolamine can increase its metabolism in nontumor cells. In an in vivo study, Berger et al. (2017) demonstrated that the administration of melatonin increases glutamine in brain tissue of rats with brain damage due to hypoxia. On the other hand, Martins, Fernandes, Bartol, Cipolla-Neto, and Costa Rosa (1998) have already shown that melatonin increased glutamine metabolism in macrophages and lymphocytes of rats with depressed immune system. If this also occurred in tumor cells, it would be ineffective in terms of promoting TCA, as having blocked one of the enzymes (DLD) of the α-ketoglutarate dehydrogenase complex, the carbons from glutamine would only contribute to the accumulation of α-ketoglutarate and therefore to the inhibition of HIF-1α.

The mechanisms suggested above are only a part of those that can be involved in the regulation of the TCA cycle by melatonin in cancer cells. The effects of melatonin on this cycle; the mechanisms mediating these effects and their consequences on cell fate deserve to be further studied in depth.

As LDH activity is also diminished after treatment with melatonin—probably as a consequence of the inhibition of HIF-1α and glycolysis—the production of lactate from pyruvate may also be decreased. So, this indolamine could be able to reduce the two main pathways of energy production in these cells, that is aerobic glycolysis and oxidative phosphorylation. Collectively, these events may cause an energy crisis that seriously influence tumor growth, increases ROS and eventually lead to cancer cell death.

2.4 | Melatonin regulation of gluconeogenesis in cancer cells

The elevated expression of PCK2 by melatonin (Puente-Moncada et al., 2020) might forward pyruvate toward the reverse pathway of glycolysis, that is, gluconeogenesis. This could be a natural response to counteract the diminished uptake of glucose and the defects in its use as discussed above. However, this response would likely be inefficient, and fall in a vicious cycle where more glucose may become available but could not be used. Moreover, PCK2 could have other functions, depending on the cell type and the level of its basal expression in a tumor. Thus, it could be involved in the induction of cell death: as noted above, there are some tumor types where PCK2 overexpression induces cell death (Luo et al., 2017) or decreases chemoresistance (Park et al., 2014).

The mechanisms of regulation of PCK2 expression by melatonin have not been yet described. However, this expression is regulated by several factors that melatonin has been previously shown to regulate. Thus, the inhibition of PCK2 has been observed after the administration of substances that activate the Akt signaling pathway (Xiang et al., 2019), so Akt inhibition by melatonin could also collaborate to the increased expression of PCK2. Furthermore, among the factors that regulate the expression of PCK2 are tumor necrosis factor (TNF), which decreases its expression (Hill & McCallum, 1992) or AMPK, which increases it (Hubert, Husson, Chedeville, & Lavoinne, 2000), both previously reported as melatonin-regulated in other experimental models. This indolamine decreases the expression of TNF (Zarezadeh et al., 2019) and increases the activity of AMPK (Mi et al., 2018).

The H6PD gene codes for H6PDH, an enzyme catalyzing the two first reactions in the endoluminal PPP occurring in the endoplasmic reticulum and distinct from the cytosolic G6PDH. Its function is to produce NADPH as a reducing molecule to counteract the ROS produced in this organelle (Hewitt, Walker & Stewart, 2005). Additionally, new functions on glucose catabolism in the endoplasmic reticulum were recently described in cancer cells for H6PDH (Marini et al., 2016), although their exact significance has yet to be determined. The functions of the H6PDH rise after treatment with melatonin are not currently explained but they could be related to the cellular needs of reducing power.

2.5 | Consequences of the increase of gluconeogenesis and decrease of aerobic glycolysis on the PPP

One of the alterations produced as a consequence of gluconeogenesis is the rise in the intermediate 3PG, which inhibits 6PGDH activity (Hitosugi et al., 2012), therefore inhibiting the oxidative phase of the PPP (Liu et al., 2018). Results from Hevia et al. (2017), who found an inhibition of this pathway due to a reduction in G6PDH activity after melatonin treatment in prostate cancer cells, also points in this direction. Although 3PG does not directly inhibit G6PDH, inhibition of an enzyme downstream in the pathway could cause the upstream enzymes to also be inhibited by excess of product. The few previously existing studies on the regulation of G6PDH by melatonin are carried out in experimental models of diabetes (Sudnikovich et al., 2007). In these models, melatonin exerts protection against diabetic oxidative stress and a weak action on

G6PDH. The mechanisms of action of said regulatory effect of the indolamine on this enzyme were not studied. In these models, melatonin seems to exert the opposite effect to that described in prostate cancer cells (Hevia et al., 2017), which is to be expected, as in prostate cancer cells this indolamine induces cell death, while diabetes falls within a large pool of diseases in which melatonin exerts cytoprotection. Besides, G6PDH can be directly activated by HIF-1α (Gao, Mejías, Echevarría, & López-Barneo, 2004), so the inhibition of HIF-1α by melatonin could inhibit the PPP. Although direct action of melatonin on G6PDH should be explored, these data suggest that the regulation exerted by melatonin in cancer cells on G6PDH is not direct, but a consequence of the metabolic alterations triggered after its administration. Puente-Moncada et al. (2020) did not find changes in the expression of this enzyme by RT-PCR; however, they did not test their samples for G6PDH activity or posttranscriptional regulation. In summary, whereas obviously further studies are needed, melatonin could inhibit the PPP, a pathway proposed as a promising target for new antitumoral agents (Patra & Hay, 2014). This alteration, together with the decrease of TCA cycle metabolic flux mentioned above, may be the origin of high ROS production after the exposure of these cells to melatonin.

2.6 | Melatonin regulation of glycogenolysis in cancer cells

Glycogenolysis is another means to obtain energy in the cell. Tumor cells exhibit increased glycogen synthesis induced by hypoxia; this is a survival mechanism (Pelletier et al., 2012). Sanchez-Sanchez et al. (2015) had observed abundant accumulations of glycogen in Ewing's sarcoma cells, where melatonin also induces cytotoxicity and inhibition of aerobic glycolysis. Glycogen accumulations disappeared after 4 hr of treatment with melatonin. This could again be a survival strategy by cells facing a reduced energy supply due to the inhibition of various metabolic routes by melatonin. Reduced glycogen stores have also been observed in other tumors where melatonin exerts a cytotoxic effect (Batista et al., 2014).

In summary, as shown in Figure 4, melatonin attacks at least four metabolic pathways previously reported to be therapeutic targets in cancer. There is a reduction of both aerobic glycolysis and metabolic flux in the TCA cycle after treatment with melatonin in cancer cells in which this indolamine results cytotoxic. Such alterations may cause a shortage of energy and an ROS rise and consequently cell death. Prostate cancer cells also showed inhibition of the PPP while LMA cells with the FLT3-ITD mutation presented activation of glycogenolysis and gluconeogenesis. It is possible that gluconeogenesis could also be involved in the induction of cell death. Further studies on TCA, gluconeogenesis, and the PPP deserve attention in both cancer types.

3 | CONCLUSIONS

Tumor metabolism is currently one of most active fields of cancer research, with aerobic glycolysis being a hallmark of cancer. The search of drugs targeting enzymes involved in this process and in the metabolic pathways directly linked to it such as the TCA cycle, PPP, and gluconeogenesis is a challenge in cancer research. Recently, it has been shown that high concentrations of melatonin inhibit both aerobic glycolysis and the carbon flux through the TCA cycle in cancer cells where it also induces cell death. Indeed, this indolamine inhibits the PPP which, together with the inhibition of the TCA cycle, may account for the increase of ROS previously reported in the cells in which this indolamine induces cell death. Finally, it activates the mitochondrial enzyme PCK2, the first enzyme of gluconeogenesis together with its cytosolic counterpart, which is enough to induce cell death in some cancer cells (cells where it is not overexpressed). Thus, melatonin targets the glucose metabolic pathways currently recognized as therapeutic targets in cancer. Bearing in mind that melatonin is a molecule without relevant side effects, further studies addressing the resolution of the existing gaps on its mechanism of action are required.

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AUTHOR CONTRIBUTIONS

C. R. conceived and prepared the writing and drafted the manuscript. P.-M. N., S.-S. A., T.-C. M., D.-O. C., and I. A. contributed in the search and analysis of information. V. M. prepared the figures. V. M. and F. H. contributed to the critical review of the manuscript. R. R., F. H., and R.-B. J. reviewed and analyzed the final version of the article. All authors read and approved the final version of the work to be published.

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