Melatonin: An important anticancer agent in colorectal cancer

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1 INTRODUCTION

Colorectal cancer is one of the most clinically challenging cancers, with an increased incidence expected with the current aging population of the world. Colorectal cancer (CRC) can have various signs and symptoms based on the anatomical location, cancer stage, and tumor progression (Astin, Griffin, Neal, Rose, & Hamilton, 2011; Darband et al., 2018; Farooqi, Roche, Djamgoz, & Siddik, 2019). Based on the genes differently expressed, CRC is divided into four subtypes of CMS1–4, and a fifth mixed subtype (Inamura, 2018). Each one of these subclasses have their own unique set of mutations, which means each subgroup of tumors present a unique alteration in normal signaling pathways, such as metabolic dysregulation, WNT and MYC activation, KRAS and APC mutations and more, which enable them to have different clinical characteristics, such as having a specific anatomical location (left vs. right colon), have varying potentials for metastasis and angiogenesis, and have varying degrees of sensitivity to conventional chemotherapy agents (Baek, 2017).
These differences also contribute to the overall prognosis of colorectal cancer, and scholars have suggested that further understanding these differences could be a guidance for the use of the therapeutic options available (Baek, 2017). More important, some agents that modulate cellular signaling pathways have been proposed as means to increase sensitivity to medication and to modulate the aforementioned signaling pathways in a way that favors anticancer phenotypes (Mehta, Murillo, Nairnath, & Peng, 2010). Natural compounds have gained attention in this regard, including polyphenols (Darband, Kaviani, & Yousefi, 2018), Retinoids, natural acidic compounds and more (Ko & Moon, 2015). Melatonin is one of these natural compounds, which has had a preserved role in evolution (Schomerus & Korf, 2005). This molecule was initially sensitized in prehistoric bacteria, enabling them to withstand oxidative stress, but gained more sophisticated roles in more sophisticated creatures (Reiter, Tan, & Galano, 2014). This molecule is best known because of its pivotal role in the circadian rhythm and mediating the physiologic changes that happen according to the day-night cycle (Grivas & Savvidou, 2007) and setting seasonal rhythms in mammals, and contributing to their accompanying changes in physiologic functions (Claustrat & Leston, 2015). Melatonin is mainly synthesized by the pineal gland in an intricate enzymatic pathway in which tryptophan is hydroxylated, decarboxylated to form serotonin, which itself undergoes acetylation and methylation to form melatonin (Schomers & Korf, 2005). Melatonin synthesis in mammals is dependent on the day-night cycle, as in instances of reduced light exposure, norepinephrine is released from sympathetic nerve endings, causing an increase in the intracellular levels of c-AMP, causing the activation of protein kinase A, which in turn regulates the function of arylalkylamine N-acetyltransferase, the rate-limiting enzyme in melatonin production (Schomers & Korf, 2005).

Melatonin is also capable of modulating many signaling pathways essential for cellular functions (Luchetti, Canonico, & Betti, 2010), such as cellular metabolism, DNA damage response (DDR), cell to cell communication, and more. melatonin exerts its function by affecting its receptors, and also by acting as a direct antioxidant agent. Melatonin receptors are G protein-coupled receptors that have three subtypes of MT-1, MT-2, and MT-3. The first two receptors are found in humans and have specific distribution in the body. The MT1 is expressed in the pars-tuberalis of the pituitary gland, the suprachiasmatic nuclei (SCN) of the hypothalamus, the skin, and the retina. This receptor is involved in the inhibitory effect of melatonin on mammalian brains, as it alters the function of the SCN, a master regulator of brain function (Emet et al., 2016). The MT-2 receptor is expressed in osteoblasts, vessels of extremities, and the retina. This receptor is mainly active in the phase shifting of the circadian clock (Emet et al., 2016; Maria et al., 2018). Melatonin also affects nuclear receptors termed retinoid Z receptors or retinoid orphan receptors. This family of receptors comprises of three subgroups, the alpha subgroup which is found in the liver, skin, thymus and the brain, the beta subgroup which is only found in the retina and the brain and the gamma subgroup which is found in the thymus, muscles, testis, liver, heart and the pancreas (Zhang, Luo, Wu, & Xu, 2015). These receptors play important roles in lipid and glucose metabolism, immune regulation, neurogenesis, bone maintenance and more less studied functions (Jetten, 2009; Zhang et al., 2015).

Recently, the role of melatonin in cancer has gained attention. Numerous studies have shown that melatonin is able to interfere in signaling pathways essential for cancer progression by affecting its receptors, and also by acting as an antioxidant agent. This has been further shown by studies examining the expression of melatonin receptors on colorectal cancer cell lines and figuring that invasive cancer cells lines have decreased expression of melatonin receptors that correlate with bad prognosis.

Currently, more effort is being invested in developing strategies to utilize melatonin as a common therapeutically option, both to increase success rates of therapy and also to reduce the unwanted consequences of chemotheraphy and radiotherapy (Lissoni, Rovelli, Brivio, Fumagalli, & Brera, 2008; Monobe, Hino, & Sumi, 2005).

2 | MELATONIN FUNCTION IN THE LOWER GUT

Unlike the central nervous system, the role of melatonin in the intestine is less well known (Chen, Fichna, Bashashati, Li, & Storr, 2011). Different distribution of melatonin receptors in the gut and the greater amount of it in the intestine compared to the brain indicates the importance of melatonin in the intestinal function (Bubenik & Brown, 1997). Here are some of these melatonin functions in the gastrointestinal tract (Figure 1). It is noteworthy to mention that colorectal cancer is partly related with abnormal colorectal function, as many studies have shown that individuals with distinct defects in colorectal function are at increased risk for cancer.

Melatonin may be able to enhance normal colorectal function and thus have a protective role against cancer (Chen et al., 2011).

2.1 | Motility

Melatonin is able to alter the bowel movements by affecting its receptors (Lucchelli, Santagostino-Barbone, & Tonini, 1997). Drago, Macauda, & Salehi (2002) showed that intraperitoneal injection of melatonin in rats increases intestinal movements. Importantly, Melatonin changes the pattern of preprandial and postprandial intestinal movements due to an inhibition in the irregular spiking activity of migratory motor complexes (Merle et al., 2000). Harlow & Weekley (1986) showed that melatonin injection, despite the lack of effect on the frequency of intestinal movements, reduced the strength of contractions. Melatonin, by affecting the apamin-sensitive potassium channels, inhibits smooth muscle contraction of the intestine (Reyes-Vázquez, Naranjo-Rodríguez, García-Segoviano, Trujillo-Santana, & Prieto-Gómez, 1997). Also, Melatonin reduces intestinal movements and prolongs colon transit time in humans (Lu, Song, Gwee, & Ho, 2009). The effect of melatonin on bowel movement is important because ir-regularities in bowel movement is associated with conditions, such as diverticulosis, hemorrhoids, and...
probably colorectal cancer, as a study had found that self-reported constipation was associated with a moderate increase in the risk of colorectal cancer (Tashiro et al., 2011).

2.2 | Secretions of the bowel

Although melatonin is not secreted from the gut, it plays an important role in the intestinal function by affecting intestinal secretion. It has been shown that using melatonin in rats with inflammatory bowel disease reduces diarrhea (Cuzzocrea et al., 2001). Melatonin, with its effect on the secretion of chloride, causes changes in the intestinal secretion content (Chan, Lui, Wong, & Poon, 1998). It also reduces ion discharge in rat distal colon by inhibiting prostaglandin E2 and sodium nitroprusside (Mrinka, Hock, Rybová, & Pácha, 2008). As previously understood, conditions associated with abnormalities in colon secretions are a risk factor for colorectal cancer, including inflammatory bowel diseases (Axelrad, Lichtiger, & Yajnik, 2016).

2.3 | Inflammation

Melatonin plays a key role in stabilizing the intestinal mucosa and preventing inflammation (Ercan, Çetinel, Contuk, Çikler, & Şener, 2004). In a study, it turned out that melatonin reduces the inflammatory processes involved in colitis by unknown mechanisms (Nosálová, Zeman, Černá, Navarová, & Zakálová, 2007). Later it became clear that Melatonin has a protective effect against colitis by reducing the matrix metalloproteinase-2 and matrix metalloproteinase-9 (Esposito et al., 2008). Localized reduction of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 due to the use of melatonin results in anti-inflammatory effects on experimental colitis (Dong, Mei, & Yu, 2003). Reducing the proinflammatory processes by NF-kappa B inhibition is another way in which melatonin acts through it (Li, Yu, & Yu, 2005; Majidinia, Alizadeh, & Yousefi, 2017). The protective role of melatonin with its antiapoptotic effects is another mechanism that has been reported in the treatment of colitis in animal models (Akcan, Kucuk, & Sozuer, 2008). Melatonin also reduces the activity of macrophages by reducing the activity of IL-1, TNF-alpha, and NO (Mei et al., 2002). These studies are clinically significant because of the fact that one of the most important conditions predisposing individuals to cancer is inflammatory bowel disease, which is characterized by a dysregulation in the immune response in the colon (Matricon, Barnich, & Ardid, 2010). And melatonin is now being considered as a therapeutic option in these patients, with clinical trials showing favorable results (Mozaffari & Abdollahi, 2011). Interestingly, more recent studies are being concentrated on the role of melatonin and the day–night cycle and sleep on the flare-up of such conditions, and early studies are suggesting that such relations may, in fact, exist (Swanson, Burgess, & Keshavarzian, 2011).

2.4 | Microbiota

Microbiota affects the metabolic and physiologic processes of the intestine, which makes it very important for intestinal health (Jandhyala, Talukdar, & Subramanyam, 2015). It has been shown that melatonin is effective in altering the normal intestinal flora, thereby contributing to the regulation of secretion, motility, and immune functions of the intestine (Yin et al., 2018). Human circadian rhythms with melatonin secretion make changes in the microbiota of the intestine and thus have a regulative effect (Paulose, Wright, Patel, & Cassone, 2016). Melatonin has a positive effect on the development, immunization and intestinal health of infants by regulating the normal flora of the intestines (Anderson, Vaillancourt, Maes, & Reiter, 2016). A study by Zhu, Ma, Ding, Jiang, & Fang (2018) was able to find that melatonin contributed to the increase in the number of Firmicutes in mice, suggesting a possible role for melatonin in preventing chronic inflammation in the bowel.
3 | MELATONIN DYSFUNCTION IN COLORECTAL CANCER

As mentioned before and as it will be discussed in detail, melatonin has many anticancer effects. One of these is the stabilization of clock genes which if disrupted, may promote cancer. A study by Momma had found that dysregulation of clock genes in human specimens of colorectal adenomas and carcinomas was associated with increased tumor size and invasion (Momma et al., 2017).

More so, many case-control studies have demonstrated the importance of the day and night cycle and sleep in cancer progression (Thompson et al., 2011). A recent case-control study found that there was a meaningful increase in the risk of colorectal cancer among patients who reported sleep disturbances (Lin, Liu, Wang, Chung, & Chien, 2019). A study by Schernhammer et al. found that nurses who worked for three nights and more a month for more than 15 years were at an increased risk of colorectal cancer. The authors suggested that the increase in cancer risk may be in part because of reduced melatonin production, as it was previously noted that 2 weeks of exposure to nighttime light significantly decreased melatonin production (Schernhammer et al., 2003). Interestingly, previous studies had found that patients with colorectal cancers had decreased levels of melatonin in their plasma compared with normal individuals (Vician et al., 1999). Studies on patients with sleep problems and also night shift workers found that these individuals had a decreased levels of urinary excretion of melatonin-related molecules, such as 6-sulphatoxymelatonin, highlighting its essential role in the circadian cycle (Davis & Mirick, 2006). Altogether, it seems that abnormal melatonin secretion which could be the result of abnormal sleep habits or cause of sleep disturbances may have a significant role in promoting the carcinogenesis of colorectal cancer.

4 | ANTICANCER EFFECTS OF MELATONIN: GENERAL MECHANISMS

4.1 | Effects on cell signaling pathways

As mentioned, disruption in normal cell signaling pathways is commonly seen in cancer. Melatonin is able to act on many signaling pathways by affecting their main mediators (Figure 2). Studies have listed some of the most significant of these signaling pathways, including DDR, PI3K/AKT pathway, NF-κB pathway, MEK/ERK pathway, the angiogenesis pathway (VEGF-HIF-1alpha) and many more (Cheng et al., 2019; Kim, Kim, & Yoo, 2014; Luchetti, Betti, & Canonico, 2009). DDR is one of the most important of these pathways. DDR is a complicated chain of molecules involved in sensing of DNA damage, recruiting appropriate molecules to the DNA damage site and initiating multiple downstream signaling pathways that repair DNA damage, initiate apoptosis, enter the cell into senescence or cause cell cycle arrest (Giglia-Mari, Zotter, & Vermeulen, 2011). Many known mutations that lead to cancer occur in genes related to the DDR cascade, including ATM, ATR, BRCA1 and BRCA2, 53BP1, RPA, and p53. P53 is a central regulator of downstream DDR effectors, which determines the cells ultimate fate after DNA damage (Mirza-Aghazadeh-Attari et al., 2018; Reinhardt & Schumacher, 2012). It is well understood now that melatonin is able to modulate multiple mediators and transducers of DDR, and have anticancer effects in cells (Majidinia et al., 2017). Interestingly, melatonin also mediates effectors involved in downstream functions such as apoptosis and DNA repair. This has important implications in treating cancer models, as inducing apoptosis and limiting DNA repair after DNA damage that has been implicated via radiation and chemotherapy are therapeutic goals to reduce resistance to treatment (Asghari, Ghobadi, Moloudizargari, Fallah, & Abdollahi, 2018). More will be discussed regarding the many possibilities which arise with the use of melatonin.

Another important determinant in the prognosis of cancers is the emergence of metastasis. The mechanisms by which melatonin inhibits cancer metastasis are mentioned in many studies and some of them include upregulation of E-cadherin in the breast cancer (75), reduced expression or activity of MMP-9 in gastric cancer (76) and downregulation of endothelin-1 in colon cancer through the inactivation of FoxO-1 and NFκB (77). All of these are made possible by melatonin’s ability to target signaling pathways involved in cellular proliferation, migration, metabolism, and angiogenesis (Gonçalves et al., 2016). Another effect of melatonin on suppressing cancer cell metastasis is through mechanisms related to epithelial-to-mesenchymal transition (EMT) of cancer cells. EMT is a mechanism that potentiates cancer cells to invade more and increase the rate of metastasis of tumors. This phenomenon happens through the inhibition of the NF-κB pathway and C/EBPβ suppression by melatonin (Wu et al., 2016).

![Figure 2](image-url) The effects on melatonin on various signaling pathways involved in cancer

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**References:**

- Davis, T., & Mirick, D. (2006). Abnormal sleep habits or cause of sleep disturbances may have a significant role in promoting the carcinogenesis of colorectal cancer. *Cancer*. 106(12), 2816-2823.
- Thompson, W., & Mirick, D. (2011). Abnormal sleep habits or cause of sleep disturbances may have a significant role in promoting the carcinogenesis of colorectal cancer. *Cancer*. 117(13), 3230-3236.
- Wu, C., & Mirick, D. (2016). Abnormal sleep habits or cause of sleep disturbances may have a significant role in promoting the carcinogenesis of colorectal cancer. *Cancer*. 117(13), 3230-3236.
Melatonin also inhibits metastasis by affecting PI3K/AKT signaling and toll-like receptors (TLRs) (Nefedova & Gabrilovich, 2007). TLR mediated activation of PI3K pathway cause an elevation of metastasis and invasion of ovarian cancer via the production of galectin-1, and melatonin as a TLR inhibitor can inhibit this process (Chuffa, Fioruci-Fontanelli, & Mendes, 2015). Melatonin also inhibits pathways, such as WNT/β-catenin, Notch1, Jak/Stat, MAPK, and the Hedgehog pathways, which are all important pathways in promoting cellular proliferation, epithelial–mesenchymal transformation, and uncontrolled cellular proliferation (Mayo et al., 2017; Motilva, García-Mauriño, Talero, & Illanes, 2011; Nefedova & Gabrilovich, 2007; Zheng et al., 2017).

### 4.2 Effects on tumor microenvironment

Malignant cells are situated in a cluster of nonmalignant cells, including fibroblasts, immune cells, the extracellular matrix, and normal cells belonging to the original tissue of which cancer has risen. There is a complicated relationship between all of these cells and interactions made between these cells is detrimental for the progression of cancer (M. Wang et al., 2017). Melatonin is able to modulate the tumor microenvironment, both by affecting cell signaling, and also having immune-modulatory roles and acting as an antioxidant. Melatonin also reduces angiogenesis, which limits the amount of energy and oxygen the tumor receives. The combined effect is that tumors are less aggressive and tend to a reduction in the rate of metastasis (Bondy & Campbell, 2018; Maschio-Signorini et al., 2016). Sonehara et al. (2019) showed that melatonin had an important role in regulating tumor aggressiveness in acidosis conditions, conditions which happen regularly at the tumor microenvironment.

### 4.3 Antioxidant effect

As mentioned melatonin has indirect effects via its receptors and direct effects as an antioxidant molecule. Melatonin’s antioxidant role may be its most preserved one, as it is used widely in primitive organisms, such as bacteria (Zhao et al., 2019). Melatonin acts as a direct detoxificant agent for substances, such as nitric oxide, peroxyl radical, peroxyiminate, and hydroxyl radicals. It also modulates redox enzymes, such as catalase, lipooxygenase, paraoxonase, and superoxide dismutase (Reiter, Mayo, & Tan, 2016). This antioxidant effect is especially important in guarding the cell against environmental factors. Many stimuli, such as UV light, exposure to various toxins and many byproducts of normal cellular metabolism are potentially toxic for the cell, as they can damage the DNA of the cell, interfere with the function of the mitochondria and sabotage enzymatic pathways. Studies have shown that cells that are exposed to melatonin have reduced rates of mutations in contact with radiation and show reduced rates of DNA damage (Anisimov, Popovich, & Zabehzinski, 2006).

### 4.4 Regulating immune function

Melatonin receptors are widely distributed on multiple organs and cells of the immune system including peripheral blood mononuclear cells, T and B lymphocytes, monocytes, the thymus, the spleen and promyelocytes. Studies have found that melatonin affects the basal function of the immune system, and regulates while it is stimulated. Studies have shown that in basal conditions melatonin increases the count of lymphocytes, natural killer cells, macrophages, neutrophil count and chemotactic movements, and the size of the spleen. These effects may be beneficial in preventing the surge of malignant cells early in their appearance (Carrillo-Vico, Lardone, Álvarez-Sánchez, Rodríguez-Rodríguez, & Guerrero, 2013). This effect may be best shown by modulation of NK cell function by melatonin. These cells are responsible to destroy malignant cells and are produced in response to IL-2, IL-6, and IL-12, all which melatonin may increase their levels. Also, melatonin affects T helper cells 1 and increases the production of IFN-gamma, a potent stimulant of immune function especially cellular immunity (Srinivasan, Pandi-Perumal, Brzezinski, Bhatnagar, & Cardinali, 2011).

But contrary to its role in basal immune function, melatonin is able to dampen immune response in instances of chronic immune diseases and pathologic over stimulation of the immune system, as it can reduce the expression of immune mediators, such as IL-1beta, IL-6, tumor necrosis factor (TNF) alpha, and the levels of enzymes, such as MMP-9, MMP-2, and amylase (Srinivasan, Maestroni, & Cardinali, 2005). One immune disease of interest is autoimmune colitis. Various forms of autoimmune colitis including Crohn’s disease and ulcerative colitis are conditions strongly linked to colorectal cancer, and melatonin may have a beneficial effect in patients with these conditions, but not by its positive effect on the immune system, but rather by its role in inhibiting it.

### 5 Therapeutic effects of melatonin in colorectal cancer

#### 5.1 Role in apoptosis and autophagy

One early mechanism by which cancer cells are able to proliferate uncontrollably and acquire new mutations is by evading apoptosis, which is a physiological mechanism to eliminate cells with damage to their DNA that cannot be repaired (Ahmad Farooqi, Damiano Gadaleta, Ranieri, & Fayyaz, 2017). It is now known that apoptosis may be performed in two distinct yet connected pathways of intrinsic and extrinsic apoptosis. Further, recently a new pathway has been suggested, the perforin/Granzyme pathway (Fayyaz, Javed, & Attar, 2019). The intrinsic pathway is initiated by the formation of mitochondrial transmembrane potential pores, leading to loss of transmembrane potential and the release of proapoptotic molecules, such as Smac/DIABLO, cytochrome C, and the serine protease HtrA2/Omi that activate a set of molecules called caspases (Shahwar et al., 2019). Caspase 9 is the first caspase activated in the intrinsic apoptosis pathway. Cytochrome c activates Apaf-1 and causes the
clustering of pro-caspase 9, ultimately resulting in the formation of apoptosome. Another set of molecules released from the mitochondria translocate to the nucleus and cause DNA fragmentation. These molecules mediate the changes witnessed in the nucleus during the apoptosis process and are themselves activated by caspases, namely caspase 3 (Fulda & Debatin, 2006). The extrinsic apoptosis pathway is initiated by the activation of extracellular transmembrane receptors belonging to the TNF receptor superfamily. These receptors are activated by molecules such as FasL and TNF-alpha. In this pathway, caspase 8 is activated by a formation of proteins called death-inducing signaling complex (DISC), and itself activated downstream caspases, such as caspase 3, 6, and 7 (Hongmei, 2012). The third pathway of apoptosis is the perforin/ Granzyme pathway. In this pathway, cytotoxic T lymphocytes use membrane perforatin proteins called perforin to secret granules that include serine proteases called granzyme A and B. These enzymes will activate caspase 10, which activates caspase 3, which is the initiation of the execution cascade and is utilized by all three pathways (Elmore, 2007).

It has been suggested that melatonin can potentiate its anticancer effects, in part by promoting apoptosis. In a study by Hong et al. (2014) the effects of melatonin were examined on HCT116 CRC cells. It was found that melatonin administration resulted in an interplay of apoptosis, senescence, and autophagy (Table 1).

A study by Yun, Kim, Lee, & Lee (2018) found that administration of melatonin to colorectal cells was able to induce apoptosis by suppressing the levels of cellular prion protein (PrPC) and PTEN-induced kinase 1 (PINK1). PINK1 one is a serine/threonine-protein kinase with important roles in the regulation of the function of mitochondria, namely enabling cells to endure mitochondrial stress. Melatonin’s effect on the expression of PINK1 resulted in mitochondrial-mediated apoptosis, and interestingly, knockdown of PrPC potentiated this effect (Yun et al., 2018). This is significant because other studies have shown that chemoresistant CRC cells, such as those of SNU-C5/Oxal-R have increased expression of PrPC, and that cotreatment with melatonin and conventional chemotherapy agents, such as oxaliplatin, could increase the rate at which cell death and apoptosis happened (Lee, Yoon, Han, Yun, & Lee, 2018).

One other mechanism by which melatonin is able to induce apoptosis is by affecting the levels of calcium ions in the cytosol. Calcium is an important mediator of multiple signaling pathways, including apoptosis, and depletion of calcium from the endoplasmic reticulum is related to cellular stress. Melatonin is able to target the type 1 sodium/calcium exchanger and type 1 IP3 receptor and induce endoplasmic reticulum stress, causing increased apoptosis. noteworthy, melatonin has innate antioxidant effects, which would contradict the aforementioned effects, but it is witnessed that this antioxidant ability is less significant in tumor cells compared to nontumor cells (Chovancova et al., 2017).

Autophagy is a process closely linked to apoptosis and cellular senescence in which the cell recycles unnecessary cellular compartments in response to cellular stress. Autophagy is a stepwise process where an isolation membrane expands and turns into a phagophore that eventually turns in to an autophagosome, which connects with lysosomes and degrades its inner contents (Mizushima, 2007). Autophagy has contrary roles in cancer progression. It can result in apoptosis, and reduce the progression rate towards cancer, and also enable cancer cells to avoid apoptosis by better adjusting with the environmental conditions, acting as a cancer-promoting mechanism (Yun & Lee, 2018). Interestingly, autophagy takes part in an intricate cross-linking between cellular senescence and apoptosis, which is in part regulated by DNA damage response (Pawlowska, Szczepanska, Szatkowska, & Blasiak, 2018). Melatonin is an important regulator of autophagy and both inhibits and stimulates autophagy. These functions are mediated by affecting master regulators of upstream signaling cascades of autophagy including NF-KB, PI3K/AKT signaling, Hif2, nuclear respiratory factor (NRF) and forhead box O (FOXO; Mirza-Aghazadeh-Attari et al., 2019). Hong et al. (2014) have reported that melatonin increased the expression of key mediators of autophagy, such as Beclin-1 in HCT-116 colorectal cancer cells, and resulted in the induction of senescence. This effect was mediated via the inhibition of AKT signaling (Hong et al., 2014). Unfortunately, not enough is known about the intricate relation between autophagy and other DNA damage response endpoints in colorectal cancer, but looking at other cancers and the current knowledge regarding autophagy’s role in cancer progression, more will be known about how to view autophagy in colorectal cancer, and how to target it to reduce cancer progression.

5.2 | Role in cell proliferation

Uncontrolled cellular proliferation is the driving force of malignant formation in cancerous cell lines, enabling the expansion of the tumor mass, cellular invasion into adjacent organs, increased malignant potential, and increased distant metastasis (Evan & Vousden, 2001). Cellular proliferation is such an important indicator of the malignant potential that it is as a prognostic indicator of numerous cancers (Adams et al., 2016). Furthermore, almost every other mechanism involved in the promotion of cancer progression eventually contributes to sustained cellular proliferation (Feitelson et al., 2015). The centerpiece which controls cellular proliferation is a network of molecules termed the cyclin-dependent kinases and their associating molecules, such as p21, p27, p57, p16, p15, and more that together form checkpoints in the cell cycle, limiting the potential for uncontrolled proliferation, and also acts as a gateway towards cellular arrest, senescence, and apoptosis (Collins, Jacks, & Pavletich, 1997). The chronical gap initiated because of the activity of these molecules also enables the cell to initiate DNA repair process, which enables cells to evade malignant transformation (Branzei & Foiani, 2008). Many cellular pathways exert direct and indirect effects on the cell cycle, regulating its pace and functionality, such as Wnt/β-catenin signaling, Notch signaling, insulin-like growth factor signaling and other growth factor signaling pathways, NF-κB signaling, Hedgehog signaling, DNA damage response pathway, PI3K signaling being the most important (Duronio & Xiong, 2013; Karimian et al., 2018; Mirza-Aghazadeh-Attari et al., 2018; Valdespino-Gómez, 2008).
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<th>Melatonin concentration</th>
<th>Molecules involved in anticancer effect</th>
<th>Major effect</th>
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<tr>
<td>Induction of apoptosis</td>
<td></td>
<td></td>
<td>By suppressing PINK1 and PrPc, melatonin increases the formation of superoxide radicals, leading to cellular stress and apoptosis.</td>
<td>Yun et al., 2018</td>
</tr>
<tr>
<td>SNU-C5/WT</td>
<td>1 mM</td>
<td>PrPc – PINK1</td>
<td></td>
<td></td>
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<tr>
<td>SNU-C5/Oxal-R</td>
<td>500 µM</td>
<td>PrPc</td>
<td>A decrease in the levels of PrPc was witnessed in cotreatment with oxaliplatin and melatonin</td>
<td>Lee, Yun et al., 2018</td>
</tr>
<tr>
<td>DLD-1</td>
<td>10 µM</td>
<td>type 1 sodium/calcium exchanger and type 1 IP3 receptor</td>
<td>Melatonin-induced apoptosis by targeting the regulation of intracellular calcium.</td>
<td>Chovancova et al., 2017</td>
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<tr>
<td>Cellular proliferation</td>
<td></td>
<td></td>
<td>NO is synthesized in increased amounts in colorectal cancer cell lines and promote mitosis. Melatonin inhibited the formation of NO.</td>
<td>García-Navarro et al., 2007</td>
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<tr>
<td>HT-29</td>
<td>4 mM</td>
<td>Nitric oxide (NO)</td>
<td></td>
<td></td>
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<tr>
<td>HT-29</td>
<td>2 mM</td>
<td>CCK-A receptor</td>
<td>Combined treatment with CCK-A inhibitors and melatonin reduced the proliferation of CRC cells.</td>
<td>González-Puga et al., 2005</td>
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<tr>
<td>CT-26</td>
<td>3 mM</td>
<td>N/A</td>
<td>Doses of melatonin more or equal to 3 m-M were able to decrease the proliferation of CRC cell lines.</td>
<td>Farriol, Venereo, Orta, Castellanos, &amp; Segovia-Silvestre, 2000</td>
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<td>HCT-116</td>
<td>1 µM</td>
<td>microRNA-24</td>
<td>Melatonin inhibited the expression of miRNA-24 which targeted DNA damage response genes and resulted in reduced proliferation of CRC cell lines.</td>
<td>Mori et al., 2016</td>
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<tr>
<td>S707</td>
<td>500 µM</td>
<td>PrPc - Oct4</td>
<td>Combined treatment inhibited the Oct-4 axis, resulting in reduced metastasis and invasion.</td>
<td>Lee, Yun et al., 2018</td>
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<td>RKO</td>
<td>25 µM</td>
<td>P38- MAPK</td>
<td>Melatonin altered the signaling of p38-MAPK and resulted in decreased expression of light chain myosin kinase.</td>
<td>Zou et al., 2015</td>
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<td>RKO</td>
<td>2.5 mM</td>
<td>ROCK- p38-MAPK-ZO-1</td>
<td>Melatonin increased the expression of cell junction proteins (ZO-1), and limited cell migration and invasion.</td>
<td>Liu et al., 2017</td>
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<tr>
<td>HT-29</td>
<td>1 mM</td>
<td>N/A</td>
<td>Melatonin increased sensitivity to 5-FU and increased apoptosis.</td>
<td>Pariente, Bejarano, Rodriguez, Pariente, &amp; Espino, 2018</td>
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<td>HCT116, SW480, COLO320, DLD-1, HT 29, RKO, CaCO2, and SW620</td>
<td>1–4 mM</td>
<td>thymidylate synthase- miR-215-5p</td>
<td>Melatonin increased the expression of miR-215-5p, which targets thymidylate synthase.</td>
<td>Sakatani et al., 2018</td>
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(Continues)
Valdespino-Castillo, & Valdespino-Castillo, 2015). Early studies have shown the beneficial effect of melatonin in reducing cellular proliferation caused by cancer progression and in response to the external stimulus of the aforementioned signaling pathways (Cos, Garcia-Bolado, & Sanchez-Barcelo, 2001; Zhang et al., 2017). More recent studies have further highlighted these mechanisms in colorectal cancer cell lines. A study by García-Navarro et al. (2007) found that melatonin inhibited the proliferation of HT-29 cell line by affecting the nuclear receptors of melatonin, and by reducing the amount of nitric oxide produced by HT-29 cells. Gonzalez-Puga et al. (2005) added that melatonin potentiated the antiproliferative effects of devazepide, lorglumide, and proglumide (all cholecystokinin [CCK] receptor antagonists), and could be a suitable option for combined therapy. Farriol et al. (2000) investigated the effects of melatonin on CT-26 murine cancer cells and found that melatonin at doses equal to 3 mM significantly reduced the proliferation of cancer cells (a 47% reduction was seen), but lower doses of melatonin were not able to significantly reduce cell division. Interestingly, these results were not dependent on estrogen receptors, and was also not dependent on damage to the cell lines, as levels of lactate dehydrogenase remained stable. As mentioned, cellular signaling pathways are important effectors in cell proliferation, and these pathways are themselves regulated by microRNAs, and it has been shown that particular microRNAs enable malignant transformation, such as microRNA-24. This microRNA increases the proliferation rate of cancer cells increases the rate of migration and metastasis, and also cause a dysregulation in the normal balance of gene translation, such as those involved in DNA damage response. Mori et al. (2016) showed that long term treatment with melatonin was able to reverse the effects of microRNA-24 and reduce the amount of microRNA in a posttranscriptional manner.

More is being suggested regarding the role of melatonin receptors on the proliferation of colorectal cancer cells. As mentioned, decreased expression of these receptors is coupled with increased cellular proliferation and increased rates of invasion, and numerous colorectal cancer cell lines have decreased expression of MT receptors. It is thought that melatonin may exerts its antiproliferative effects on normal colon cells by its receptors, and lack of them enables cells to proliferate uncontrollably. More is to be discovered regarding the role of melatonin and its receptor-mediated action in colorectal cancer, especially the role of melatonin in reducing cellular proliferation in human colon cancer specimens (Gil-Martin, Egea, Reiter, & Romero, 2019).

### 5.3 Role in metastasis and cell invasion

Invasion and metastasis are two hallmarks of malignant neoplasms, which determine the ultimate clinical outcome of the patient, as many individuals with cancer receive palliative care due to inoperable tumors invading vessels or key organs, or having metastatic lesions. The process of invasion and metastasis is characterized by stepwise changes in cancer cells, including the change in cellular adhesion molecules, epithelial-mesenchymal transformation, intravasation and
its accompanying changes for cell survival and finally arrest at the distal organs and formation of micrometastatic masses and metastatic colonization (Asghari et al., 2018). These changes are accompanied, and most probably are directly caused by alterations in cell signaling pathways which govern important functions, such as survival, metabolism, gene expression regulation, such as PI3K/AKT/m-TOR signaling, Wnt/β-catenin signaling, VEGF signaling, HGF/Met signaling, ERK signaling, Ras signaling, and most probably others (Beelen et al., 2014; Colombo, Maciel, Ferreira, Da Silva, & Zuccari, 2016; Ward et al., 2001). Numerous studies have shown that melatonin, in fact, is effective in modulating the aforementioned pathways, and can hypothetically be useful in preventing metastasis in CRCs. More evidence in this regard was found by scholars comparing the expression of melatonin receptors (MT1, MT2, and ROR-alpha) in CRCs. It was shown that invasion was higher in CRCs (FHC, Caco-2, DLD-1, and HT-29 cell lines) expressing fewer degrees of these receptors, and that nonselective agonists were able to reduce cell invasion (Léon et al., 2012). More in this regard was shown in a study by Casado et al. (2017) where the correlation between the expression of MT1 and MT2 with stem-cell markers was assessed. They found that CRCs with increased expression of CD44 and CD66 had lower levels of MT1 and MT2 expression, and ultimately were of advanced stages. Lee, Yun et al. (2018) investigated the effects of melatonin combined with 5-fluorouracil on colon cancer stem cells. It was found that there was a significant correlation between the PrPC and Oct4 axis and the metastasis and higher tumor stage. Melatonin treatment effectively inhibited this axis and hampered its signaling. This was accompanied by an inhibition of cellular markers such as Oct4, Nanog, Sox2, and ALDH1A1. Treatment also resulted in significantly reduced cell invasion, metastasis, proliferation, and angiogenesis. Zou et al. (2015) showed that melatonin treatment inhibited the migration of the RKO cancer cell line, by regulating the expression of Myosin Light Chain Kinase (MLCK). This was probably mediated by the inhibition in the phosphorylation of p38, a member of the MAPK family with established roles in the promotion of cellular proliferation and metastasis (Wada et al., 2017). Similar results were also reported by Liu et al. (2017) with the addition that they found that melatonin treatment also increased the expression of zonula occludens-1 and immunofluorescence assay showed its increased presence in the tight junctions, reducing the probability of distant metastasis.

5.4 | Role in drug resistance

Therapy for colorectal consists of a combination of chemotherapy, radiation therapy, and surgery, with chemotherapy being widely used for tumors of different stages. Conventional chemotherapy for colorectal cancer has consisted of use of platinum agents and 5-FU, with more novel agents, such as cetuximab, regorafenib, irinotecan, capecitabine, and bevacizumab being considered more recently for therapy (Sartore-Bianchi et al., 2009; Watanabe et al., 2012). These agents act on colorectal cancer cells by different means, such as damaging the cellular DNA, causing the activation of the DDR and thus initiation of apoptosis, or by affecting signaling pathways related to EGFR and VEGF, which promote cancer progression and cellular proliferation (Cheung-Ong, Giaever, & Nislow, 2013). Importantly, as mentioned, melatonin is able to modulate many upstream signaling pathways affected by this mediation and thus has been used to reduce resistance against them (Asghari et al., 2018). In a systemic review, Y. Wang et al., 2012 showed that use of melatonin increased 1-year survival, and increased tumor-remission and reduced side effects of chemotherapy in concurrent use with chemotherapy regimens in solid tumors, including colorectal cancer. Pariente et al. suggested that treatment with melatonin in doses of 1 m-M increased the sensitivity to 5-FU in HT-29 cells, which was shown by an increase in the activation of caspase 3 after combined treatment (Pariente et al., 2018).

Furthermore, melatonin is able to diminish resistance to treatment by novel means. A study by Sakatani, Sonohara, and Goel (2018), found that melatonin helped overcome resistance to 5-FU by downregulation of thymidylate synthase in a variety of CRC cell lines. Interestingly, this effect was coupled with an increase in microRNA-215-5p, which directly targets thymidylate synthase. Lee, Yun et al. (2018) suggested that melatonin reduced resistance to oxaiplatin CRC SNU-C5/Oxal-R by reducing the expression of the cellular prion protein, causing an increase in apoptosis, induction of endoplasmic reticulum stress, and increasing superoxide generation. Previous studies had shown that prion protein is involved in cellular proliferation, metastasis, and resistance to treatment, by having wide interactions with multiple signaling pathways (Santos, Lopes, & Martins, 2015). Fic et al. suggested that melatonin increased sensitivity to doxorubicin in part by increasing the expression of P-glycoprotein. This protein is coded by the AABCB1 gene and is responsible for the transmembrane transportation of toxins, drug, and other substances. Melatonin was able to increase the expression of this protein, and potentiate the effect of doxorubicin (Fic et al., 2017).

5.5 | Role in radiation resistance

Radiation therapy is used in a wide area of cancers including colorectal cancer, both as palliative treatment and as a curative treatment in conjunction with chemotherapy and surgery. Many scholars have tried to decipher how tumors gain resistance to radiotherapy, and prominent hypothesis has emerged, such as the role of mutation in DNA damage response genes, the role of cancer stem cells, the role of reactive oxygen species, and others (Barker, Paget, Khan, & Harrington, 2015; Diehn & Clarke, 2006; Narumi, 2003). As melatonin can have meaningful effects on the tumor microenvironment, the DNA damage response cascade and the oxidative status of the cell, it is considered a key molecule in increasing sensitivity to radiation (Majidinia et al., 2017). Q. Wang et al. (2018) found that a combination of ionizing radiation and melatonin had beneficial effects in vivo and in vitro. In vitro, melatonin decreased the expression of genes involved in double stranded DNA damage repair and promoted the caspase-mediated...
apoptotic response. It also caused cellular arrest in G2/M, which was essential for its effects in promoting sensitivity to radiotherapy.

6 | MELATONIN IN IN VIVO AND HUMAN STUDIES

A number of studies have aimed to examine the effect of melatonin in animal models, and have found interesting results. Melatonin targeted almost all of the pathways which had been shown to be affected in in vitro studies, including the apoptosis pathways, cellular proliferation, and the immune system. Mice and rats were mainly studied, and doses of melatonin administration ranged from 1 microgram to 25 micrograms, and therapy was continued from 6 days up to 6 months in some studies. All studies used per Os or subcutaneous injection for delivery of melatonin (Gil-Martin et al., 2019). Altogether, enough evidence exists to suggest that melatonin could be used in humans, but serious questions remain regarding dosage and duration of treatment and optimal delivery of drug in human subjects, as no clinical study has been done in colorectal cancer patients, which specifically examines the effect of melatonin on the progression of cancer (Gil-Martin et al., 2019). Most studies performed on the human subject focus on melatonin’s effects in inducing sleep, and suggest that melatonin be given in doses up to 6 mg orally (Costello, Lentino, & Boyd, 2014). A systemic review of clinical trials of melatonin found that most studies reported time to maximal plasma concentration of 50 min and an elimination half-life of 45 min, and a bioavailability of 15%. This study concluded that some important parameters such as maximal plasma/serum concentration, clearance, volume of distribution area-under-the-curve plasma/serum concentrations were reported with variations between studies and no consensus could be made (Harpøe, Andersen, Gögenur, & Rosenberg, 2015).

7 | PERSPECTIVES

Melatonin has gained much attention as an agent with capability in chemoprevention of cancers, and it is now widely recognized that melatonin could be utilized as supportive therapy in multiple cancers (Grant, Melan, Latimer, & Witt-Enderby, 2009; Tamtaji, Mirhosseini, Reiter, Behnamfar, & Asemi, 2019). Compared with other cancers with significant disease burden, such as breast cancer, limited studies are preformed and many aspects of melatonin use in colorectal cancer is not yet been covered by scientific publications. These include the role of melatonin in modulating physiologic functions of the bowel and cancer risk, and there are no studies aiming specifically to understand the underlying cellular pathways that play a role in melatonin’s function in the bowels. Importantly, although the importance of the circadian rhythm is known in cancer progression, it is not well understood if a disruption in these genes has any significant effect on the anticancer effects of melatonin, or that melatonin supplementation could limit the procarcinogenic effect. It is also not well understood whether each of the mentioned has a direct role in suppressing cancer in humans or that both melatonin and the clock genes act in conjunction via similar pathways. the prospect of using melatonin as combination therapy with novel antiangiogenesis medication, novel chemotherapy agents, and immunotherapy regimens. Another issue which is not yet fully studied is the drug delivery of melatonin. Novel studies are suggesting interesting methods, such as use of chitosan nanoparticles, liposomes, and transdermal or transmucosal patches to deliver melatonin, but these methods are not compared comprehensively in any published study yet, and also it is not known if local administration of melatonin will have the same effects as systemic delivery (Hafner, Lovric, Pepic, & Filipovic-Grcic, 2011; Lee, Parrott, Ayres, & Sack, 1994; Permuy, López-Peña, González-Cantalapiedra, & Muñoz, 2017).

8 | CONCLUSION

In this review, the importance of melatonin in colorectal cancer was discussed. Melatonin is an agent that has important regulatory effects on the most important functions of the gastrointestinal system and is now being considered as an agent to prevent colorectal pathologies. One of these is colorectal cancer, which is related to inflammatory conditions in the colon. More so, the dysfunctional cellular signaling involved in colorectal cancer promotes cellular proliferation, migration, and metastasis, and also promotes resistance to medication and radiotherapy. Melatonin is effectively able to inhibit cell proliferation, limit cellular migration, and thus prevent distant metastasis, and is also able to regulate signaling pathways that contribute to resistance against medication in the first place. Currently, studies suggesting a beneficial role for melatonin are at their early stages and no large scale clinical trials are published regarding the effects of melatonin on colorectal cancer. Future investigates will determine the extent to which melatonin is considered as standard therapy in colorectal cancer.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

AUTHOR CONTRIBUTIONS

M. M. A. A., A. M., S. M., S. G. D., and S. S. focused on the basic aspects of the review and wrote the article. A. A. prepared figures, B. Y. reviewed the state of the art on the topic, M. M. guided the present scientific team, wrote and revised the article. All the authors studied and approved the final manuscript.

DATA AVAILABILITY

The data used to support the findings of this study are included in the article.
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