The role of melatonin in colorectal cancer treatment: a comprehensive review

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Abstract: Colorectal cancer (CRC) is one of the most common types of cancer worldwide, known as the second leading cause of cancer-related deaths annually. Currently, multimodal treatment strategies, including surgical resection, combined with chemotherapy and radiotherapy, have been used as conventional treatments in patients with CRC. However, clinical outcome of advanced stage disease remains relatively discouraging, due mainly to appearance of CRC chemoresistance, toxicity, and other detrimental side effects. New strategies to overcome these limitations are essential. During the last decades, melatonin (MLT) has been shown to be a potent antiproliferative, anti-metastatic agent with cytotoxic effects on different types of human malignancies, including CRC. Hence, this comprehensive review compiles the available experimental and clinical data analyzing the effects of MLT treatment in CRC patients and its underlying molecular mechanisms.

Keywords: anticancer drug combinations, colorectal cancer, melatonin, review

Introduction
Colorectal cancer (CRC) is one of the primarily responsible causes for cancer-related deaths worldwide.1-3 If diagnosed in the early stage, the 5-year survival rate after curative surgery reaches about 90%, dropping to only 14% with the appearance of distant metastasis.4 Multimodal treatment regimens combine surgery with neo-adjuvant/adjuvant chemotherapy (CTx) techniques as well as targeted therapies using antibodies and kinase inhibitors.5 This results in decided improvement of survival for metastatic disease patients reaching a median of nearly 30 months.6

CRC liver metastases (CRLM) occur in 20–70% of CRC patients, and represent the major cause of death in this group.7,8 Surgical resection is the standard treatment for patients with resectable CRLM (when it is possible to achieve R0 resection while maintaining at least a 30% functional liver tissue) resulting in a 5-year survival rate of up to 58% when combined with CTx.9,10 However, CRC cells are known to lose susceptibility to CTx by various mechanisms.11,12 To optimize treatment strategies, further evaluation of promising drug combinations and additives is necessary for improve patient survival.

Melatonin (MLT), a natural body hormone, previously demonstrated impressive protective properties against toxic effects of CTx and radiotherapy, in both experimental and clinical studies.13-15 This could pave the way for application of higher doses of CTx, resulting in improved efficacy.16 Moreover, MLT itself exerts antiproliferative, antimetastatic, and cytotoxic effects on different types of human malignancies, including CRC.17-19 Taking into consideration that this endogenously generated molecule lacks any moderate–severe side effects at even relatively high dose,20-22 currently renders MLT a trending research topic, particularly in cancer treatment studies (Figure 1).

The objective of this comprehensive review is to summarize literature on the role of MLT in CRC and CRLM treatment, and discuss the mechanisms of its anti-cancer properties, based on experimental studies and clinical trials.
Melatonin

The endogenous hormone MLT, also known as N-acetyl-5-methoxytryptamine, was discovered by the North American dermatologist, Aaron Lerner in 1958 at Yale University. For many years, MLT was considered to be synthesized exclusively in the pineal gland from the amino acid tryptophan in response to darkness (Figure 2). However, the presence of MLT-related enzymes was subsequently uncovered in a number of extrapineal tissues such as the gastrointestinal tract, thymus, spleen, heart, muscle, and others. Moreover, MLT was also identified in most living organisms, including bacteria, macroalgae, plants, invertebrates, and mammals.

In humans and mammals, two classes of plasma membrane associated MLT receptors are known. They are named MT1 (encoded by MTNR1A gene) and MT2 (encoded by the MTNR1B gene), respectively, and are expressed in various parts of the central nervous system and the peripheral organs. Another binding site for MLT, MT3, has been recently characterized as a...
MLT-sensitive form of the quinone reductase. It is related to the cell’s xenobiotic metabolism (detoxification enzyme) expressed in various tissues. However, MT3 is an entirely different type of protein, not fully satisfying the criteria for MLT receptors. MLT also binds to cytoplasmic proteins, like the calcium-binding proteins calmodulin or tubulin, as well as nuclear receptors like RORα/RZR, and acts through non-receptor-mediated mechanisms.

Soon after MLT was discovered to have a direct free radical scavenger effect, it was proposed as an anti-cancer agent. In 2004, MLT was shown to be associated with mechanisms influencing cancer initiation and cell growth for the first time. Since then, numerous studies have supported these findings.

Modes of action in anti-cancer treatment

**Proliferation inhibition**
MLT exerts a wide range of antiproliferative properties by inhibition/blocking of the cancer cell cycle under *in vitro* and *in vivo* conditions. Activation of MT1 and MT2 receptors inhibits adenyl cyclase and cyclic adenosine monophosphate, leading to a reduction in uptake of linoleic acid, which serves as an energy source for tumor growth and tumor growth-signalling molecules. MLT-induced inhibition of linoleic acid uptake is considered as antiproliferative mechanism, and was described by Blask et al. in a rat hepatoma model. Furthermore, antiestrogenic effects, and the ability to inhibit tumor growth by reducing glucose uptake and modifying the expression of the GLUT1 transporter have been shown *in vitro* and *in vivo*. In a study on a murine colon carcinoma-derived cell line, MLT inhibited tumor growth in a dose-dependent manner; DNA synthesis was inversely associated with MLT dose. Moreover, Lee et al. demonstrated that histone deacetylase 4 plays a crucial role in MLT-induced apoptosis in LoVo (a human colon adenocarcinoma cell line) cells, most likely through the inactivation of calcium/calmodulin-dependent protein kinase (CaMK) IIα. More recently, Lee et al. showed that MLT influences apoptosis and autophagy in human colon cancer stem cells by regulating the cellular prion protein (PrPC)-octamer-binding transcription factor (Oct) 4 axis. Additionally, MLT acts *via* B-cell lymphoma 2 (Bcl-2) expression, the c-Jun N-terminal kinase, p38 and nuclear factor (NF)-κB-p65 signalling pathways, thereby promoting apoptosis in different types of cancer.

**Apoptosis activation**
Resistance to apoptosis is one of the fundamental hallmarks of cancer. There is strong evidence that MLT enhances and promotes apoptosis in various tumor cells. Jia-Yi Wei et al. demonstrated that histone deacetylase 4 plays a crucial role in MLT-induced apoptosis in LoVo (a human colon adenocarcinoma cell line) cells, most likely through the inactivation of calcium/calmodulin-dependent protein kinase (CaMK) IIα. More recently, Lee et al. showed that MLT influences apoptosis and autophagy in human colon cancer stem cells by regulating the cellular prion protein (PrPC)-octamer-binding transcription factor (Oct) 4 axis. Additionally, MLT acts *via* B-cell lymphoma 2 (Bcl-2) expression, the c-Jun N-terminal kinase, p38 and nuclear factor (NF)-κB-p65 signalling pathways, thereby promoting apoptosis in different types of cancer.

**Angiogenesis inhibition**
As neovascularization is essential for tumor growth and metastasis, controlling angiogenesis is a promising treatment option for limiting cancer progression. Angiogenesis is regulated by factors like vascular endothelial growth factor or hypoxia induced factor (HIF), and MLT has the ability to regulate the oncogenic potential by controlling the expression of such factors. In *in vitro* and *in vivo* (rodent models) studies demonstrated that MLT affects HIF-1α, the most important and primary transcriptional mediator in hypoxic response, in a receptor-independent manner. Previous findings suggest that upregulation of microRNAs mediates MLT induced anti-angiogenic effects in breast and hypoxic prostate cancer cells *in vitro*. These findings have been approved in a xenograft model.

**Modulation of the immune system**
The immune system presents the greatest potential for the specific destruction of malignant cells not harming normal tissue, and with the long-term memory offering a potent opportunity to prevent cancer recurrence. The immuno-enhancing action of MLT was evident in recent animal and clinical studies. MLT has been shown to contribute to effective anti-cancer immune responses *via* mechanisms such as stimulation of interleukins (IL-2, IL-6, IL-12) production, the inhibition of macrophage-mediated suppressive events, and inflammatory status modulation.
**Antioxidative and pro-oxidative effects**

MLT and its metabolites exert antioxidative effects. Besides direct scavenging of reactive oxygen and nitrogen species (ROS/RNS), MLT stimulates antioxidant enzymes, suppresses pro-oxidant enzymes, and improves mitochondrial function, thereby reducing radical formation in physiological and pharmacological concentrations. In *in vitro* studies demonstrated a role of MLT in the maintenance of levels of the intracellular antioxidant glutathione, which has been related to cancer cell growth. Elevated levels of ROS/RNS have been detected in almost all cancer entities, where they promote aspects of tumor development and progression. For example, the steady-state levels of superoxide are significantly higher (5- to 20-fold) in colon cancer cell lines compared with normal colon epithelial cells and fibroblasts. Interestingly, a few *in vivo* studies found that MLT induces the generation of ROS at pharmacological concentrations (μM to mM range) in tumor cells, leading to the assumption that MLT could be a conditional pro-oxidant. This property of MLT may promote an inflammatory response leading to apoptosis in tumor cells, but further *in vivo* studies are needed to concretize this scenario.

**Effects of MLT on CRC**

Epidemiological studies demonstrated that night-shift workers might have an increased risk for cancer development, including CRC. This finding may support the hypothesis that environmental light inhibits MLT production, resulting in cancer promotion. In *in vitro* and *in vivo* studies have shown that MLT exerts anticancer effects on CRC. Those studies are compiled in Tables 1 and 2, respectively.

**The synergistic effect of MLT and anti-cancer drugs in CRC treatment**

For several years, scientists searched for strategies to reduce the toxic side effects of CTx on the one hand, and to increase tumor-specific response on the other. Data on the synergistic effects of CTx agents and MLT on CRC suggest that MLT should be used in therapeutic concentrations rather than its physiological concentrations, which lack sufficient protection of cells from the toxic effects of CTx. So far, most of these studies were performed *in vitro*, lacking confirmation *in vivo*.

**In vitro studies**

*In vitro* studies evaluating MLT synergistic effects with anti-cancer drugs in CRC treatment are compiled in Table 3. It seems that addition of MLT increased the specific cytotoxicity of anti-cancer drugs, including doxorubicin, irinotecan, cisplatin, oxaliplatin, and 5-fluorouracil (5-FU), on different CRC cell lines, including drug resistant cells. However, MLT was not effective in inducing DNA damage in healthy human cells. The main mechanisms suppressing tumor growth, proliferation, and tumor-mediated angiogenesis include (a) apoptosis activation through simultaneous modulation of cytochrome c/caspase, matrix metalloprotease 9 (MMP9)/cyclooxygenase 2 (COX-2), and p300/NF-κB signalling pathways; (b) suppression of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) and nuclear factor kappa B (NF-κB)/inducible nitric oxide synthase (iNOS) signalling pathways; and (c) downregulation of PrPC.

**In vivo studies**

There are a limited number of *in vivo* studies evaluating the synergistic effects of MLT combined with anti-cancer drugs in CRC treatment (Table 4). Authors found that octreotide and MLT administered separately exert antiproliferative and proapoptotic effects on CRC in a murine model; however, combination of substances did not show additive effects. Recently, Bakalova et al. investigated the anticancer effect of MLT in combination with active irinotecan metabolites in a murine model of CRC. This combined treatment reduced tumor volume by decreasing oncogenic and increasing onco-suppressive ROS in tumor tissue. However, the small sample size of the study limits its explanatory power.

**Clinical studies**

The first controlled clinical trial to evaluate the effects of MLT on cancer was published in 1987 by Lissoni et al. A total of 19 patients suffering from advanced solid tumors, including CRC, not responding to standard therapies, were included in the study. MLT was administered intramuscularly at a daily dose of 20 mg, followed by a maintenance period with lower MLT doses in patients with remission, a stabilization of disease or an improvement in performance status. MLT induced an amelioration of the performance status score and the quality of life in 60% patients. This preliminary study suggested a promising
In 1990, Barni et al. evaluated the therapeutic activity of the pineal hormone MLT in patients with metastatic CRC who did not respond to 5-FU treatment. MLT was administered intramuscularly at a daily dose of 20 mg for 2 months, followed by daily oral administration of 10 mg. An evident improvement in performance status was seen only in 5 out of 14 (36%) patients. The results indicated a lack of antitumor activity for MLT in treatment of cancer patients not responding to standard anti-cancer therapies.

### Table 1. Summary of in vitro studies investigating the effects and mechanisms of MLT on CRC.

| Authors            | Subject            | Dose of MLT | Effect and mechanism                                                                                                                                 |
|--------------------|--------------------|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| Pawlikowski et al. | Colon 38 cells     | 10^{-7} and 10^{-9} M | Data support the hypothesis of the involvement of the RZR/RORα nuclear sites in the oncostatic action of MLT.                                    |
| Farriol et al.     | CT-26 cells        | 1, 2 and 3 mM | Decrease in cell growth was attributed to a moderate, but significant, antiproliferative action of MLT on this non-hormone-dependent cell line.          |
| Winczyk et al.     | Colon 38 cells     | 10^{-3} M   | The direct oncostatic effect of MLT depends on MT2 and RZR/RORα nuclear receptors activity.                                                         |
| Garcia-Navarro et al. | HT-29 cells     | 10^{-3} M   | Reduction of nitric oxide production by cultured HT-29 cells seem to be directly dependent on the oncostatic properties of MLT.                   |
| Winczyk et al.     | Colon 38 cells     | 10^{-7} and 10^{-9} M | Membrane MLT receptors are not indispensable to the oncostatic action of MLT, and, thus, other pathways such as nuclear signalling and receptor-independent mechanism may be also involved. |
| Park et al.        | HCT-116 cells      | 1 mM        | MLT suppresses tumor angiogenesis by inhibiting HIF-1α stabilization under hypoxia.                                                                  |
| Liu et al.         | HCT-15 cells       | 1 nM        | MLT enhances DNA repair capacity probably by affecting genes involved in DNA damage responsive pathways.                                           |
| Hong et al.        | HCT-116 cells      | 10 μM       | MLT activates cell death programs and induces G1-phase arrest at the advanced phase.                                                               |
| Batista et al.     | Caco-2 cells       | 1.56 and 0.78 μg/ml | MLT promotes cytotoxicity in Caco-2 cells, which can probably be related to the generation of ROS.                                               |
| Leon et al.        | Caco-2 and T84 cells | 1 mM        | MLT reduces endothelin-1 expression and secretion in colon cancer cells through the inactivation of FoxO-1 and NF-κB.                            |
| Zou et al.         | RKO cells          | 25 μM       | MLT inhibits the migration of colon cancer cells by down-regulating myosin light chain kinase expression through cross-talk with p38 MAPK.        |
| Wei et al.         | LoVo cells         | 1 mM        | MLT induces apoptosis of CRC cells through HDAC4 nuclear import mediated by CaMKII inactivation.                                                   |
| Buldak et al.      | HCT-116 cells      | 10^{-6} M   | MLT treatment increases ROS levels and decreases cellular viability.                                                                               |
| Liu et al.         | RKO cells          | 2.5 mM      | MLT inhibits colon cancer cell migration by downregulating Rho-associated protein kinase expression via the p38/MAPK signalling pathway.        |
| Chovancova et al.  | DLD1 cells         | 0.1, 1, and 10 μM | MLT is able to induce apoptosis in cancer cells through the type 1 sodium/calcium exchanger, and type 1 IP3 receptor.                         |
| Yun et al.         | SNU-C5/WT cells    | 1 mM        | MLT induces mitochondria-mediated cellular apoptosis in CRC cells via a PrPC-dependent pathway.                                                   |

CaMK, calcium/calmodulin-dependent protein kinase; CRC, colorectal cancer; FoxO, forkhead transcription factors O; HDAC, histone deacetylase; HIF, hypoxia-inducible factor; IP3, inositol trisphosphate; MAPK, mitogen-activated protein kinase; MLT, melatonin; MT, melatonin receptor; PrPC, cellular prion protein; ROR, retinoid receptor-related orphan receptor; ROS, reactive oxygen species; RZR, retinoid Z receptor.
MLT in metastatic CRC patients resistant to 5-FU treatment.

Promising synergistic anti-cancer effects of MLT and IL-2 have been demonstrated in a study including 35 patients with various tumors, that is, CRC, gastric cancer, hepatocellular carcinoma, or pancreas adenocarcinoma. Oral administration of 50 mg MLT daily started 7 days prior to IL-2 administration, resulting in an overall response rate of 23%. Another study suggested that preoperative neuro-immunotherapeutic treatment with low-dose IL-2 and MLT (40 mg/day) is a well-tolerated therapy, able to prevent surgery induced lymphocytopenia in cancer patients. A decline in lymphocyte number greater than 30% occurred in 8/10 control patients, but only in 1/10 treated with IL-2 and MLT.

A large clinical study in 2002 included 1440 patients with untreated advanced solid tumors (279 patients with CRC), receiving supportive care alone or supportive care in combination with MLT to investigate the effect of MLT on cancer. The second part of this study, evaluated the influence of MLT on the efficacy and toxicity of CTx in 200 metastatic patients with CTx-resistant tumors (51 patients with CRC). CTx regimen consisted of 5-FU with folinic acid (FA) or raltitrexed. Additionally, MLT (20 mg/day) was administered orally during the night. The results led to the assumption that MLT may be effective in the prevention of cancer progression-related symptoms, such as cachexia, asthenia, and lymphocytopenia, and CTx-induced toxicity, such as thrombocytopenia, asthenia, and neurocardiotoxicity. Moreover, the study revealed synergistic effects of MLT in combination with anti-cancer drugs.

### Table 2. Summary of in vivo studies investigating the effects and mechanisms of MLT on CRC.

| Authors          | Subject | Dose of MLT                           | Effect and mechanism                                                                 |
|------------------|---------|--------------------------------------|--------------------------------------------------------------------------------------|
| Anisimov et al.  | Rats    | 20 mg/l in water, PO; 5 days/week 6 months | MLT demonstrated an inhibitory effect on DHM-induced intestinal carcinogenesis by preventing a decrease in numbers of MLT-containing cells. Moreover, multiplicity of colon cancer was reduced. |
| Pawlikowski et al. | Mice  | 10 and 100 μg/animal, SC; 6 days     | Data support the hypothesis of the involvement of the RZR/RORα nuclear sites in the oncostatic action of MLT. |
| Kossoy et al.    | Rats    | 20 mg/l in water, PO; 5 days/week 6 months | Anti-carcinogenic effects of MLT are related to increased numbers of CD8+ lymphocytes and Fas-positive T cells. |
| Winczyk et al.   | Mice    | 10 and 100 μg/animal, SC; 6 days     | Data suggest the involvement of RZR/RORα receptors in the pro-apoptotic effect of MLT. |
| Anisimov et al.  | Rats    | 1 μg/animal, SC; 5 days/week 6 months* | Synthetic pineal peptide Epitalon showed an inhibitory effect on DMH-induced colon carcinogenesis. |
| Winczyk et al.   | Mice    | 25 μg/animal, SC; 10 days            | Nuclear RZR/RORα receptors participate in the oncostatic action of MLT. |
| Winczyk et al.   | Mice    | 25 μg/animal, SC; 6 days             | The direct oncostatic effect of MLT depends on MT2 and RZR/RORα nuclear receptors activity. |
| Kossoy et al.    | Rats    | 1 μg/animal, SC; 5 days/week 6 months* | Epitalon significantly inhibited mitotic activity of tumor cells in a model of DMH-induced carcinogenesis. |
| Kannen et al.    | Rats    | 10 mg/kg, IP; 14 days                | MLT potentially controls malignant lesions in colon tissue possibly by an early action on pericryptal colonic stroma changes, mainly upon the CD68(+) and CD133(+) cell clusters. |
| Trivedi et al.   | Mice    | 1 mg/kg, PO; 8 and 18 weeks          | MLT treatment decreased the progression of colitis-associated colon carcinogenesis by down regulating autophagy via the expression of Beclin-1, LC3B-II/LC3B-I ratio and p62. |

*These studies used synthetic pineal peptide Epitalon.
CRC, colorectal cancer; DMH, dimethylhydrazine; LC, light chain; MLT, melatonin; MT, melatonin receptor; PO, per oral administration; ROR, retinoid receptor-related orphan receptor; RZR, retinoid Z receptor; SC, subcutaneous administration.
Cerea et al. evaluated the influence of a concomitant administration of MLT on irinotecan therapeutic activity in metastatic CRC.\textsuperscript{114} The study included 30 metastatic CRC patients progressing after at least one previous chemotherapeutic line containing 5-FU. After randomization, MLT was administered orally at 20 mg/day during the night. The percent of disease-control achieved in patients concomitantly treated with MLT was significantly higher than that observed in those treated with CTx alone, at 85.7% and 43.8%, respectively.

More recently, a study randomized 370 cancer patients to receive CTx treatment alone or CTx combined with orally administered MLT (20 mg/day).\textsuperscript{14} CRC patients accounted for one-third of the study population (122 patients) treated by

### Table 3. Summary of in vitro studies investigating the synergistic effect of MLT combined with anti-cancer drugs in CRC treatment.

| Authors          | Cell line                  | Treatment                                                                 | Results                                                                                                                                                                                                                     |
|------------------|----------------------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Granzotto et al. | LoVo and LoVo/ADR          | MLT [10–2000 pg/mL] + Doxorubicin [0.3 and 10 μM]                        | The cytotoxicity of doxorubicin on sensitive and resistant cell lines slightly increased by MLT.                                                                                                                               |
| Gonzalez-Puga et al. | HT-29                | MLT [1 mM] + Lorglumide [25 μM]                                           | MLT and cholecystokinin antagonists control human colon cancer cell growth in culture, and, in combined therapy, significantly increases their efficiency.                                                                  |
| Wenzel et al.    | HT-29                     | MLT [1 mM] + Flavone [150 μM]                                            | MLT potentiates flavone-induced apoptosis in human colon cancer cells by increasing the level of glycolytic end products.                                                                                                   |
| Kontek et al.    | HT-29                     | MLT [50 μM] + Irinotecan [7.5, 15, 30, and 60 μM]                         | MLT modulates the genotoxic activity of irinotecan by degreasing DNA repair efficacy in cancer cells. However, not effective in inducing DNA damage in healthy human lymphocytes.                                                  |
| Wang et al.      | SW480; LoVo               | MLT [1 mM] + Ursolic acid [20 μM]                                        | Combined treatment significantly enhances inhibition of cancer cell proliferation and increases induction of apoptosis through simultaneous modulation of cytochrome c/caspase, MMP9/COX-2, and p300/NF-κB signalling pathways. |
| Gao et al.       | SW620; LoVo               | MLT [1 mM] + 5-Fluorouracil [30 μM]                                      | MLT synergizes the chemotherapeutic effect of 5-fluorouracil in colon cancer by suppressing PI3K/AKT and NF-κB/INOS signalling pathways.                                                                                     |
| Pariente et al. | HT-29                     | MLT [1 mM] + Cisplatin [20 μM]; MLT [1 mM] + 5-Fluorouracil [1 mM]       | MLT enhances CTx-induced cytotoxicity and apoptosis via MT3 receptor stimulation.                                                                                                                                           |
| Fic et al.       | LoVo, LoVo\textsubscript{dx} | MLT [1 mM] + Doxorubicin [0.9 μM]                                       | MLT intensifies the cytotoxic effect of doxorubicin in LoVo\textsubscript{dx} cells [CRC cells resistant to doxorubicin].                                                                                                |
| Pariente et al. | HT-29                     | MLT [1 mM] + Cisplatin [20 μM]; MLT [1 mM] + 5-Fluorouracil [1 mM]       | MLT increases the sensitivity of HT-29 cells to 5-fluorouracil treatment.                                                                                                                                                   |
| Lee et al.       | S707                      | MLT [500 μM] + 5-Fluorouracil [1 μM]                                      | Co-treatment with 5-fluorouracil and MLT inhibits the stem cell markers Oct4, Nanog, Sox2, and ALDH1A1 by downregulating PrP\textsuperscript{C} resulting in suppressed tumor growth, proliferation and angiogenesis.                  |
| Lee et al.       | SNU-C5; SNU-C5/Oxal-R     | MLT [500 μM] + Oxaliplatin [1 μM]                                        | Co-treatment with oxaliplatin and MLT increases endoplasmic reticulum stress and apoptosis of SNU-C5/Oxal-R (oxaliplatin-resistant CRC cells) cells via inhibition of PrP\textsuperscript{C}.                                             |

AKT, protein kinase B; ALDH, aldehyde dehydrogenase; COX, cyclooxygenase; CRC, colorectal cancer; CTx, chemotherapy; INOS, inducible nitric oxide synthase; MLT, melatonin; MMP, matrix metalloproteinase; MT, melatonin receptor; NF, nuclear factor; Oct, octamer-binding transcription factor; PI3K, phosphatidylinositol 3-kinase; PrP\textsuperscript{C}, cellular prion protein; Sox, Sex determining region Y-box.
oxaliplatin plus 5-FU and FA, or 5-FU and FA or weekly irinotecan. The overall tumor regression rate was significantly higher, and 2-year survival was significantly improved in patients receiving CTx and MLT.

Clinical pharmacokinetics of MLT
Several administration regimens for MLT have been investigated, but it is not yet clear which regimen results in the optimal pharmacologic effect. A systematic review by Harpsoe et al., including 22 studies with 359 volunteers/patients, provided important insights concerning the pharmacokinetics of exogenously administered MLT.115 This review documented a time to maximal plasma/serum concentration (Tmax) of approximately 50 min following oral immediate-release formulations of MLT. The half-life time of oral and intravenous MLT was about 45 min (ranging from 28 to 126 min). Bioavailability after oral administration was generally low (ranging between 9 and 33%) with significant intra-individual variability. It is proposed that the low bioavailability is caused by a considerable first-pass metabolism in the liver.116

Another systematic review of experimental or clinical studies investigated the pharmacokinetics of alternative administration regimen for MLT.117 In that review, intranasal administration demonstrated a higher bioavailability and Tmax compared with oral MLT, 55–94% and ranging from 2.5 to 7.8 min, respectively. Whereas the oral transmucosal regimen resulted in higher maximal plasma/serum concentrations with similar Tmax compared with oral MLT; transdermal administration of MLT yielded slow absorption and deposition of MLT in the skin. Since no side effects have been reported, MLT appears to be safe for daily doses up to 100 mg/kg.118 However, most of the studies included primarily young healthy volunteers, whereas previous studies indicated that the pharmacokinetics of MLT is affected by age, health status, and external factors, such as caffeine intake, cigarette smoking, and the use of oral contraceptives.22,119,120

Table 4. Summary of in vivo studies investigating the synergistic effect of MLT combined with anti-cancer drugs in CRC treatment.

| Authors          | Animals | Treatment                                                                 | Results                                                                 |
|------------------|---------|---------------------------------------------------------------------------|------------------------------------------------------------------------|
| Melen-Mucha et al.108 | Mice    | MLT (10 μg/animal, SC)+Octreotide (10 μg/animal, SC); 6 days              | Octreotide and MLT given separately exert antiproliferative and proapoptotic effects on colon cancer; no additive effects for the combined treatment. |
| Bakalova et al.73 | Mice    | MLT [10 mg/kg, SC]+EF24 [400 μg/kg, SC]+SN38 [10 mg/kg, SC]; 22 days     | The anticancer effect of the triple combination is accompanied by decreasing oncogenic and increasing onc-suppressive ROS. |

CRC, colorectal cancer; EF24, curcumin analog; MLT, melatonin; ROS, reactive oxygen species SC, subcutaneous administration; SN38, irinotecan active metabolite.

Conclusion
The effects of MLT alone and in combination with anti-cancer regimen have been studied in vitro and in vivo including animal models and clinical trials. Clinical trials focus mainly on advanced cancer patients, but the best MLT administration regimen for CRC treatment is still unknown and needs further research. To deepen the knowledge about the effects of MLT in CRC treatment, animal experiments to evaluate clinically important application regimen of MLT for treatment of complex CRC and CRLM are mandatory. This will pave the way for further clinical studies probably answering the question about the optimal application regimen for MLT.

In summary, there is sufficient evidence that MLT is involved in carcinogenesis, development, and progression of CRC cells by different mechanisms. Thus, further clinical trials are warranted to include MLT as a new promising therapeutic agent for CRC treatment.

Funding
The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest statement
The authors declare that there is no conflict of interest.

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References

1. Safiri S, Sepanlou SG, Ikuta KS, et al. The global, regional, and national burden of colorectal cancer and its attributable risk factors in 195 countries and territories, 1990–2017: a systematic analysis for the global burden of disease study 2017. *Lancet Gastroenterol Hepatol* 2019; 4: 913–933.

2. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. Epub ahead of print 12 September 2018. DOI: 10.3322/caac.21492.

3. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Abate D, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2017: a systematic analysis for the global burden of disease study. *JAMA Oncol* 2019; 5: 1749–1768.

4. Howlader N, Noone AM, Krapcho M, et al. SEER cancer statistics review (CSR) 1975–2016. National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/csr/1975_2016/ (2019). (Accessed 12 January 2020).

5. Bruns H, Kazanavicius D, Schultz D, et al. Glycine inhibits angiogenesis in colorectal cancer: role of endothelial cells. *Amino Acids* 2016; 48: 2549–2558.

6. Mi K, Kalady MF, Quintini C, et al. Integrating systemic and surgical approaches to treating metastatic colorectal cancer. *Surg Oncol Clin N Am* 2015; 24: 199–214.

7. Honari M, Shafabakhsh R, Reiter RJ, et al. Resveratrol is a promising agent for colorectal cancer prevention and treatment: focus on molecular mechanisms. *Cancer Cell Int* 2019; 19: 180.

8. Karoui M, Penna C, Amin-Hashem M, et al. Influence of preoperative chemotherapy on the risk of major hepatectomy for colorectal liver metastases. *Ann Surg* 2006; 243: 1–7.

9. Abdalla EK and Vauthey JN. Chemotherapy prior to hepatic resection for colorectal liver metastases: helpful until harmful? *Dig Surg* 2008; 25: 421–429.

10. Van Cutsem E, Cervantes A, Adam R, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol* 2016; 27: 1386–1422.

11. Hammond WA, Swaika A and Mody K. Pharmacologic resistance in colorectal cancer: a review. *Ther Adv Med Oncol* 2016; 8: 57–84.

12. Mirzaei H, Salehi H, Sahebkar A, et al. Deciphering biological characteristics of tumorigenic subpopulations in human colorectal cancer reveals cellular plasticity. *J Res Med Sci* 2016; 21: 64.

13. Farhood B, Goradl NH, Mortezaee K, et al. Melatonin as an adjuvant in radiotherapy for radioprotection and radiosensitization. *Clin Transl Oncol*. Epub ahead of print 22 August 2018. DOI: 10.1007/s12094-018-1934-0.

14. Lissoni P. Biochemotherapy with standard chemotherapies plus the pineal hormone melatonin in the treatment of advanced solid neoplasms. *Pathol Biol (Paris)* 2007; 55: 201–204.

15. Martinez-Campa C, Menendez-Menendez J, Alonso-Gonzalez C, et al. What is known about melatonin, chemotherapy and altered gene expression in breast cancer. *Oncol Lett* 2017; 13: 2003–2014.

16. Reiter RJ, Mayo JC, Tan DX, et al. Melatonin as an antioxidant: under promises but over delivers. *J Pineal Res* 2016; 61: 253–278.

17. Srinivasan V, Pandi-Perumal SR, Brzezinski A, et al. Melatonin, immune function and cancer. *Recent Pat Endoc Metab Immune Drug Discov* 2011; 5: 109–123.

18. Li Y, Li S, Zhou Y, et al. Melatonin for the prevention and treatment of cancer. *Oncotarget* 2017; 8: 39896–39921.

19. Wei JY, Li WM, Zhou LL, et al. Melatonin induces apoptosis of colorectal cancer cells through HDAC4 nuclear import mediated by CaMKII inactivation. *J Pineal Res* 2015; 58: 429–438.

20. Reiter RJ, Rosales-Corral SA, Tan DX, et al. Melatonin, a full service anti-cancer agent: inhibition of initiation, progression and metastasis. *Int J Mol Sci* 2017; 18: 843.

21. Meng X, Li Y, Li S, et al. Dietary sources and bioactivities of melatonin. *Nutrients* 2017; 9: 367.

22. Andersen LPH, Werner MU, Rosenkilde MM, et al. Pharmacokinetics of oral and intravenous melatonin, immune function and cancer. *Recent Pat Endoc Metab Immune Drug Discov* 2011; 5: 109–123.

23. Lerner AB, Case JD, Takahashi Y, et al. Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J Am Chem Soc* 1958; 80: 2587.

24. Acuna-Castroviejo D, Escames G, Venegas C, et al. Extrapineal melatonin: sources, regulation, and potential functions. *Cell Mol Life Sci* 2014; 71: 2997–3025.
25. Ackermann K and Stehle JH. Melatonin synthesis in the human pineal gland: advantages, implications, and difficulties. Chronobiol Int 2009; 23: 369–379.

26. Borjigin J, Wang MM and Snyder SH. Diurnal variation in mRNA encoding serotonin N-acetyltransferase in pineal gland. Nature 1995; 378: 783–785.

27. Stehle JH, von Gall C and Korf HW. Analysis of cell signalling in the rodent pineal gland: perceptors regulators of dynamic transcription in neural/ endocrine cells. Eur J Neurosci 2001; 14: 1–9.

28. Stefuli J, Hortner M, Ghosh M, et al. Gene expression of the key enzymes of melatonin synthesis in extrapineal tissues of the rat. J Pineal Res 2001; 30: 243–247.

29. Venegas C, Garcia JA, Escames G, et al. Extrapineal melatonin: analysis of its subcellular distribution and daily fluctuations. J Pineal Res 2012; 52: 217–227.

30. Arnao MB and Hernández-Ruiz J. Melatonin promotes adventitious- and lateral root regeneration in etiolated hypocotyls of lupinus albus L. J Pineal Res 2007; 42: 147–152.

31. Tan DX, Reiter R, Manchester L, et al. Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. Curr Top Med Chem 2002; 2: 181–197.

32. Ekmekcioglu C. Expression and putative functions of melatonin receptors in malignant cells and tissues. Wien Med Wochenschr 2014; 164: 472–478.

33. Vanecek J. Cellular mechanisms of melatonin action. Physiol Rev 1998; 78: 687–721.

34. Dubocovich ML and Markowska M. Functional MT1 and MT2 melatonin receptors in mammals. Endocrine 2005; 27: 101–110.

35. Slominski RM, Reiter RJ, Schlabritz-Loutsevitch N, et al. Melatonin membrane receptors in peripheral tissues: distribution and functions. Mol Cell Endocrinol 2012; 351: 152–166.

36. Pandiperumal S, Trakht I, Srinivasan V, et al. Physiological effects of melatonin: role of melatonin receptors and signal transduction pathways. Prog Neurobiol 2008; 85: 335–353.

37. Nosjean O, Nicolas JP, Klupsch F, et al. Comparative pharmacological studies of melatonin receptors: MT1, MT2 and MT3/QR2. Tissue distribution of MT3/QR2. Biochem Pharmacol 2001; 61: 1369–1379.

38. Hardeland R. Melatonin, hormone of darkness and more: occurrence, control mechanisms, actions and bioactive metabolites. Cell Mol Life Sci 2008; 65: 2001–2018.

39. Reiter RJ. Mechanisms of cancer inhibition by melatonin. J Pineal Res 2004; 37: 213–214.

40. Di Bella G, Mascia F, Gualano L, et al. Melatonin anticancer effects: review. Int J Mol Sci 2013; 14: 2410–2430.

41. Gatti G, Lucini V, Dugnani S, et al. Antiproliferative and pro-apoptotic activity of melatonin analogues on melanoma and breast cancer cells. Oncotarget 2017; 8: 68338–68353.

42. Hong Y, Won J, Lee Y, et al. Melatonin treatment induces interplay of apoptosis, autophagy, and senescence in human colorectal cancer cells. J Pineal Res 2014; 56: 264–274.

43. Blask DE, Dauchy RT, Sauer LA, et al. Melatonin uptake and growth prevention in rat hepatoma 7288CTC in response to dietary melatonin: melatonin receptor-mediated inhibition of tumor linoleic acid metabolism to the growth signaling molecule 13-hydroxyoctadecadienoic acid and the potential role of phytomelatonin. Carcinogenesis 2004; 25: 951–960.

44. Hevia D, González-Menéndez P, Quiros-González I, et al. Melatonin uptake through glucose transporters: a new target for melatonin inhibition of cancer. J Pineal Res 2015; 58: 234–250.

45. Farriol M, Venereo Y, Orta X, et al. In vitro effects of melatonin on cell proliferation in a colon adenocarcinoma line. J Appl Toxicol 2000; 20: 21–24.

46. Lee SE, Kim SJ, Youn JP, et al. MicroRNA and gene expression analysis of melatonin-exposed human breast cancer cell lines indicating involvement of the anticancer effect. J Pineal Res 2011; 51: 345–352.

47. Naeli P, Pourhanifeh MH, Karimzadeh MR, et al. Circular RNAs and gastrointestinal cancers: epigenetic regulators with a prognostic and therapeutic role. Crit Rev Oncol Hematol 2020; 145: 102854.

48. Moridikia A, Mirzaei H, Sahebkar A, et al. MicroRNAs: potential candidates for diagnosis and treatment of colorectal cancer. J Cell Physiol 2018; 233: 901–913.

49. Savardashtaki A, Shabaninejad Z, Movahedpour A, et al. miRNAs derived from cancer-associated fibroblasts in colorectal cancer. Epigenomics 2019; 11: 1627–1645.

50. Martin-Renedo J, Mauriz JL, Jorquera F, et al. Melatonin induces cell cycle arrest and apoptosis
51. Alonso-Gonzalez C, Menendez-Menendez J, Gonzalez-Gonzalez A, et al. Melatonin enhances the apoptotic effects and modulates the changes in gene expression induced by docetaxel in MCF-7 human breast cancer cells. *Int J Oncol*. Epub ahead of print 28 November 2017. DOI: 10.3892/ijo.2017.4213.

52. Lee JH, Yoon YM, Han YS, et al. Melatonin promotes apoptosis of oxaliplatin-resistant colorectal cancer cells through inhibition of cellular prion protein. *Anticancer Res* 2018; 38: 1993–2000.

53. Lee JH, Yun CW, Han YS, et al. Melatonin and 5-fluorouracil co-suppress colon cancer stem cells by regulating cellular prion protein-Oct4 axis. *J Pineal Res* 2018; 65: e12519.

54. Shafabakhsh R, Mirzaei H and Asemi Z. Melatonin: a promising agent targeting leukemia. *J Cell Biochem* 2020; 121: 2730–2738.

55. Li W, Wang Z, Chen Y, et al. Melatonin treatment induces apoptosis through regulating the nuclear factor-κB and mitogen-activated protein kinase signaling pathways in human gastric cancer SGC7901 cells. *OncoLett* 2017; 13: 2737–2744.

56. Um HJ, Park JW and Kwon TK. Melatonin sensitizes Caki renal cancer cells to kahweol-induced apoptosis through CHOP-mediated up-regulation of PUMA. *J Pineal Res* 2011; 50: 359–366.

57. Fat'hizadeh H, Mirzaei H and Asemi Z. Melatonin: an anti-tumor agent for osteosarcoma. *Cancer Cell Int* 2019; 19: 319.

58. Li W, Wu J, Li Z, et al. Melatonin induces cell apoptosis in Mia PaCa-2 cells via the suppression of nuclear factor-κB and activation of ERK and JNK: a novel therapeutic implication for pancreatic cancer. *Oncol Rep* 2016; 36: 2861–2867.

59. Shafabakhsh R, Reiter RJ, Mirzaei H, et al. Melatonin: a new inhibitor agent for cervical cancer treatment. *J Cell Physiol* 2019; 234: 21670–21682.

60. Ambasta RK, Sharma A and Kumar P. Nanoparticle mediated targeting of VEGFR and cancer stem cells for cancer therapy. *Vasc Cell* 2011; 3: 26.

61. Goradel NH, Ashghari MH, Moloudizargari M, et al. Melatonin as an angiogenesis inhibitor to combat cancer: mechanistic evidence. *Toxicol Appl Pharmacol* 2017; 335: 56–63.

62. Sohn EJ, Won G, Lee J, et al. Upregulation of miRNA3195 and miRNA374b mediates the anti-angiogenic properties of melatonin in hypoxic PC-3 prostate cancer cells. *J Cancer* 2015; 6: 19–28.

63. Lacerda JZ, Ferreira LC, Lopes BC, et al. Therapeutic potential of melatonin in the regulation of MiR-148a-3p and angiogenic factors in breast cancer. *Micron* 2019; 8: 237–247.

64. Finn OJ. Immuno-oncology: understanding the function and dysfunction of the immune system in cancer. *Am Oncol* 2012; 23(Suppl. 8): viii6–viii9.

65. Srinivasan V, Maestroni GJ, Cardinali DP, et al. Melatonin, immune function and aging. *Immun Ageing* 2005; 2: 17.

66. Mediavilla MD, Sanchez-Barcelo EJ, Tan DX, et al. Basic mechanisms involved in the anti-cancer effects of melatonin. *Curr Med Chem* 2010; 17: 4462–4481.

67. Lissoni P, Giani L, Zerbini S, et al. Biotherapy with the pineal immunomodulating hormone melatonin versus melatonin plus aloe vera in untreatable advanced solid neoplasms. *Nat Immun* 1998; 16: 27–33.

68. Zhang HM and Zhang Y. Melatonin: a well-documented antioxidant with conditional pro-oxidant actions. *J Pineal Res* 2014; 57: 131–146.

69. Schemmer P, Nickkhohagh A, Schneider H, et al. PORTAL: pilot study on the safety and tolerance of preoperative melatonin application in patients undergoing major liver resection: a double-blind randomized placebo-controlled trial. *BMC Surg* 2008; 8: 2.

70. Chen GC, Chen CY, Wang SH, et al. Melatonin sensitizes hepatocellular carcinoma cells to chemotherapy through long non-coding RNA RAD51-AS1-mediated suppression of DNA repair. *Cancers (Basel)* 2018; 10: 320.

71. Blask DE, Wilson ST and Zalatan F. Physiological melatonin inhibition of human breast cancer cell growth in vitro: evidence for a glutathione-mediated pathway. *Cancer Res* 1997; 57: 1909–1914.

72. Liou GY and Storz P. Reactive oxygen species in cancer. *Free Radic Res* 2010; 44: 479–496.

73. Bakalova R, Zhelev Z, Shibata S, et al. Impressive suppression of colon cancer growth by triple combination SN38/EF24/Melatonin: “oncogenic” versus “onco-suppressive” reactive oxygen species. *Anticancer Res* 2017; 37: 5449–5458.
74. Papantoniou K, Devore EE, Massa J, et al. Rotating night shift work and colorectal cancer risk in the nurses’ health studies. Int J Cancer 2018; 143: 2709–2717.

75. Schernhammer ES, Laden F, Speizer FE, et al. Night-shift work and risk of colorectal cancer in the nurses’ health study. J Natl Canc Inst 2003; 95: 825–828.

76. Pawlikowski M, Kunert-Radek J, Winczyk K, et al. The antiproliferative effects of melatonin on experimental pituitary and colonic tumors. Possible involvement of the putative nuclear binding site? Adv Exp Med Biol 1999; 460: 369–372.

77. Winczyk K, Pawlikowski M, Lawnicka H, et al. Effects of melatonin and melatonin receptors ligand N-[(4-methoxy-1H-indol-2-yl)methyl] propanamide on murine colon 38 cancer growth in vitro and in vivo. Neuro Endocrinol Lett 2002; 23(Suppl. 1): 50–54.

78. Garcia-Navarro A, Gonzalez-Puga C, Escames G, et al. Cellular mechanisms involved in the melatonin inhibition of HT-29 human colon cancer cell proliferation in culture. J Pineal Res 2007; 43: 195–205.

79. Winczyk K, Fuss-Chmielewska J, Lawnicka H, et al. Luzindole but not 4-phenyl-2-propionamidotetralin (4P-PDOT) diminishes the inhibitory effect of melatonin on murine colon 38 cancer growth in vitro. Neuro Endocrinol Lett 2009; 30: 657–662.

80. Park SY, Jang WJ, Yi EY, et al. Melatonin suppresses tumor angiogenesis by inhibiting HIF-1alpha stabilization under hypoxia. J Pineal Res 2010; 48: 178–184.

81. Liu R, Fu A, Hoffman AE, et al. Melatonin enhances DNA repair capacity possibly by affecting genes involved in DNA damage responsive pathways. BMC Cell Biol 2013; 14: 1.

82. Batista APC, da Silva TG, Teixeira AAC, et al. Ultrastructural aspects of melatonin cytotoxicity on caco-2 cells in vitro. Micron 2014; 59: 17–23.

83. León J, Casado J, Jiménez Ruiz SM, et al. Melatonin reduces endothelin-1 expression and secretion in colon cancer cells through the inactivation of FoxO-1 and NF-κB. J Pineal Res 2014; 56: 415–426.

84. Zou DB, Wei X, Hu RL, et al. Melatonin inhibits the migration of colon cancer RKO cells by down-regulating myosin light chain kinase expression through cross-talk with p38 MAPK. Asian Pac J Cancer Prev 2015; 16: 5835–5842.

85. Buldak RJ, Plic-Gumula K, Buldak L, et al. Effects of ghrelin, leptin and melatonin on the levels of reactive oxygen species, antioxidant enzyme activity and viability of the HCT 116 human colorectal carcinoma cell line. Mol Med Rep 2015; 12: 2275–2282.

86. Liu Z, Zou D, Yang X, et al. Melatonin inhibits colon cancer RKO cell migration by downregulating Rho-associated protein kinase expression via the p38/MAPK signaling pathway. Mol Med Rep 2017; 16: 9383–9392.

87. Chovancova B, Hudocova S, Lencesova L, et al. Melatonin-induced changes in cytosolic calcium might be responsible for apoptosis induction in tumour cells. Cell Physiol Biochem 2017; 44: 763–777.

88. Yun CW, Kim S, Lee JH, et al. Melatonin promotes apoptosis of colorectal cancer cells via superoxide-mediated ER stress by inhibiting cellular prion protein expression. Anticancer Res 2018; 38: 3951–3960.

89. Anisimov VN, Popovich IG, Shytlik AV, et al. Melatonin and colon carcinogenesis. III. Effect of melatonin on proliferative activity and apoptosis in colon mucosa and colon tumors induced by 1,2-dimethylhydrazine in rats. Exp Toxicol Pathol 2000; 52: 71–76.

90. Anisimov VN, Kvetnoy IM, Chumakova NK, et al. Melatonin and colon carcinogenesis. II. Intestinal melatonin-containing cells and serum melatonin level in rats with 1,2-dimethylhydrazine-induced colon tumors. Exp Toxicol Pathol 1999; 51: 47–52.

91. Anisimov VN, Popovich IG and Zabezhinski MA. Melatonin and colon carcinogenesis: I. Inhibitory effect of melatonin on development of intestinal tumors induced by 1,2-dimethylhydrazine in rats. Carcinogenesis 1997; 18: 1549–1553.

92. Kossoy G, Ben-Hur H, Popovich I, et al. Melatonin and colon carcinogenesis. IV. Effect of melatonin on proliferative activity and expression of apoptosis-related proteins in the spleen of rats exposed to 1,2-dimethylhydrazine. Oncol Rep 2000; 7: 1401–1405.

93. Winczyk K, Pawlikowski M and Karasek M. Melatonin and RZR/ROR receptor ligand CGP 52608 induce apoptosis in the murine colonic enzyme activity and viability of the HCT 116 human colorectal carcinoma cell line. Mol Med Rep 2015; 12: 2275–2282.

94. Anisimov VN, Khavinson VKh, Popovich IG, et al. Inhibitory effect of peptide epithalon on colon carcinogenesis induced by 1,2-dimethylhydrazine in rats. Cancer Lett 2002; 183: 1–8.

95. Winczyk K, Pawlikowski M, Guerrero JM, et al. Possible involvement of the nuclear RZR/R
96. Kossoy G, Zandbank J, Tendler E, et al. Epitalon and colon carcinogenesis in rats: proliferative activity and apoptosis in colon tumors and mucosa. *Int J Mol Med* 2003; 12: 473–477.

97. Kannen V, Marini T, Zanette DL, et al. The melatonin action on stromal stem cells within pericryptal area in colon cancer model under constant light. *Biochem Biophys Res Commun* 2011; 405: 593–598.

98. Trivedi PP, Jena GB, Tikoo KB, et al. Melatonin modulated autophagy and Nrf2 signaling pathways in mice with colitis-associated colon carcinogenesis. *Mol Carcinog* 2016; 55: 255–267.

99. Fic M, Gomulkiewicz A, Grzegrzolka J, et al. The impact of melatonin on colon cancer cells’ resistance to doxorubicin in an in vitro study. *Int J Mol Sci* 2017; 18: 1396.

100. Granzotto M, Rapozzi V, Decorti G, et al. Effects of melatonin on doxorubicin cytotoxicity in sensitive and pleiotropically resistant tumor cells. *J Pineal Res* 2001; 31: 206–213.

101. Gao Y, Xiao X, Zhang C, et al. Melatonin synergizes the chemotherapeutic effect of 5-fluorouracil in colon cancer by suppressing PI3K/AKT and NF-κB/iNOS signaling pathways. *J Pineal Res* 2017; 62.

102. Pariente R, Bejarano I, Espino J, et al. Participation of MT3 melatonin receptors in the synergistic effect of melatonin on cytotoxic and apoptotic actions evoked by chemotherapeutics. *Cancer Chemother Pharmacol* 2017; 80: 985–998.

103. Pariente R, Bejarano I, Rodriguez AB, et al. Melatonin increases the effect of 5-fluorouracil-based chemotherapy in human colorectal adenocarcinoma cells in vitro. *Mol Cell Biochem* 2018; 440: 43–51.

104. Gonzalez-Puga C, Garcia-Navarro A, Escames G, et al. Selective CCK-A but not CCK-B receptor antagonists inhibit HT-29 cell proliferation: synergism with pharmacological levels of melatonin. *J Pineal Res* 2005; 39: 243–250.

105. Wenzel U, Nickel A and Daniel H. Melatonin potentiates flavone-induced apoptosis in human colon cancer cells by increasing the level of glycolytic end products. *Int J Cancer* 2005; 116: 236–242.

106. Kontek R and Nowicka H. The modulatory effect of melatonin on genotoxicity of irinotecan in healthy human lymphocytes and cancer cells. *Drug Chem Toxicol* 2013; 36: 335–342.
117. Zetner D, Andersen L and Rosenberg J. Pharmacokinetics of alternative administration routes of melatonin: a systematic review. Drug Res 2015; 66: 169–173.

118. Carloni S, Proietti F, Rocchi M, et al. Melatonin pharmacokinetics following oral administration in preterm neonates. Molecules 2017; 22: 2115.

119. Härtter S, Nordmark A, Rose DM, et al. Effects of caffeine intake on the pharmacokinetics of melatonin, a probe drug for CYP1A2 activity. Br J Clin Pharmacol 2003; 56: 679–682.

120. Hilli J, Korhonen T, Turpeinen M, et al. The effect of oral contraceptives on the pharmacokinetics of melatonin in healthy subjects with CYP1A2g.-163C>A polymorphism. J Clin Pharmacol 2008; 48: 986–994.