Review

Role of Melatonin in Cancer Treatment

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Abstract. *Melatonin has revealed itself to be a pleiotropic and multitasking molecule. The mechanisms that control its synthesis and the biological clock processes that modulate the circadian production of melatonin in the pineal gland have been well-characterized. A feature that characterizes melatonin is the variety of mechanisms it employs to modulate the physiology and molecular biology of cells. Research has implicated the pineal gland and melatonin in the processes of both aging and age-related diseases. The decline in the production of melatonin with age is thought to contribute to immunosenescence and potential development of neoplastic diseases. Melatonin has been shown to inhibit growth of different tumors under both in vitro and in vivo conditions. There is evidence that the administration of melatonin alone or in combination with interleukin-2 in conjunction with chemoradiotherapy and/or supportive care in cancer patients with advanced solid tumors, has been associated with improved outcomes of tumor regression and survival. Moreover, chemotherapy has been shown to be better tolerated in patients treated with melatonin.*

Melatonin (N-acetyl-5-metoxy-tryptamine) (MLT) is a hormone mainly synthesized in the pineal gland but also in other parts of the body, including the gastrointestinal tract, bone marrow, eyes, lymphocytes and skin (1). It has been demonstrated that MLT is involved in the regulation of human chronobiological and endocrinological functions (2). Reports on plasma MLT concentrations among humans of different age strata have shown a decrease in MLT production with advancing age (3). The decline in circulating levels of hormones associated with ageing, among which MLT is included, have been suggested to contribute to the gradual deterioration of the immune system brought on by natural age advancement (immunosenescence).

MLT is not regarded to be hormone in the classical sense, but rather functions as a cell protector because it is not synthesized in a single organ and does not exert effects upon a specific target organ. It has been recognized that MLT is a molecule with paracrine, autocrine, and antioxidant effects, which exerts diverse receptor-dependent and receptor-independent actions (4, 5), with overall homeostatic functions and pleiotropic effects relevant to cell protection and survival (6). Moreover, MLT is known to possess potent antioxidant, immunomodulating, antiproliferative, oncostatic, and endocrine-modulating properties (2, 7). The oncostatic and tumor inhibitory effects of MLT, in a variety of experimental models and clinical conditions have been an increasing area of interest.

Mechanism of Action of MLT

MLT and its metabolites interact with intracellular proteins (calmodulin), (RZR) (ROR) family nuclear-membrane receptors, and receptors located in the cell membrane (8). Different forms of high-affinity (MT1) and low-affinity melatonin receptors (MT2, MT3) have been identified (9). MT1 and MT2 were initially designated as Mel1a and Mel1b, and later classified as MT1 and MT2 by the International Union of Basic and Clinical Pharmacology (IUPHAR) (10). MT1 and MT2 are included in the G-protein-coupled receptor (GPCR) group (a family of guanidine triphosphate-binding proteins) and share many of their amino acid sequences (11).

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Both MT1 and MT2 are involved in adenyl cyclase and cyclic AMP (cAMP) inhibition. A decrease in cAMP production reduces the uptake of linoleic acid. Linoleic acid, oxidized to 13-Hydroxy octadecadienoic acid (13-HODE) by 15-lipoxygenase, serves as an energy source for tumor signaling molecules and tumor growth. Inhibition of lineoleic acid uptake by MLT is regarded as a mechanism of its antiproliferative effects (9).

A third member of the MLT receptor family is the X-linked orphan G protein-coupled receptor (GPR50) (12), which shares 45% homology with MT1 and MT2 receptors. Its ligand and physiological function remain unclear, although an involvement in key hypothalamic functions, including regulation of the hypothalamo pituitary axes has been suggested (13). Moreover, orphan GPCRs can heterodimerize with GPCRs that have identified ligands, and by doing so, they regulate the function of the latter (14). In this respect, deletion of the large c-terminal tail of GPR50 suppressed the inhibitory effect of GPR50 on MT1 without affecting heterodimerization, indicating that this domain regulates the interaction of regulatory proteins with MT1 (15).

An MT3 receptor/binding site has also been investigated. Mass spectrometry and enzymatic data have confirmed that MT3 is quinone reductase 2 (QR2), a known detoxifying enzyme. The induction of this enzyme expression is associated with a decreased susceptibility to cancer initiation and progression (16, 17).

It has been shown that MLT has oncostatic properties on a wide variety of tumors, including prostate cancer, sarcoma, colorectal cancer, hepatocarcinoma, melanoma, neural tumors, laryngeal carcinoma, skin carcinoma, and ovarian, breast and cervical cancer (18). The oncostatic effects of MLT have been particularly studied in hormone-dependent tumors. Extensive evidence on the oncostatic activity of MLT is based on *in vitro* studies carried out in cell lines derived from human tumors and murine tumoral models. A general conclusion of these studies is that MLT inhibits cell proliferation and induces apoptosis in most tumor cell lines. Mechanisms of cancer inhibition by MLT include antioxidant effects; regulation of estrogen receptor expression and transactivation; modulation of the enzymes involved in the local synthesis of estrogens; modulation of the cell cycle, differentiation and apoptosis; inhibition of telomerase activity; antiangiogenesis; prevention of circadian disruption; activation of the immune system; and epigenetic factors. There is abundant information regarding the involved mechanisms provoking the effects of MLT on many tumor types, as well as reviews summarizing the results of research in a number of different malignant neoplasms (18-23).

The anticarcinogenic actions of MLT relate in part to its antioxidative and free radical-scavenging activity. The antiestrogenic properties of MLT depend on its ability to reduce the expression of estrogenic receptor alpha (ERα) and to inhibit the binding of the estradiol (E2-ER) complex to the estrogen response element (ERE) on DNA (18, 23). These effects are exerted through MLT binding to specific (MT1) membrane receptors (discussed more extensively in the next section). On the other hand, the inactivation of calmodulin by MLT, is another means by which this hormone may interact with the estrogen-signalling pathway (24). MLT is both a selective estrogen receptor and an enzyme modulator. Its preferential inhibition of ERα explains the oncostatic properties of MLT on estrogen-dependent tumors (18). Other mechanisms of action, including pro-apoptotic effects of MLT on tumor cells and inhibition of telomerase activity are only partially known (25-27).

MLT exerts direct antiangiogenic effects through inhibition of vascular endothelial growth factor (VEGF), as well as indirect effects by (a) inhibiting other tumor growth factors, such as epidermal growth factor (EGF), endothelin-1 (ET-1) and insulin-like growth factor 1 (IGF-1), which are strong mitogens and stimulate cancer angiogenesis (28), and (b) by neutralizing reactive oxygen species (ROS). Research on the antiangiogenic properties of MLT is of significant importance, with possible future clinical applications. MLT is also synthesized by lymphoid organs (bone marrow, thymus, lymphocytes) and is considered to be an immunoenhancer. The administration of MLT stimulates the production of natural killer cells, monocytes, leukocytes, interleukins (IL-2, IL-6, IL-12), interferon-gamma and tumor necrosis factor-alpha (TNFα) through its binding to specific membrane and nuclear receptors present in these cells (29). Finally, novel roles for MLT in epigenetic modulation of gene transcription have been suggested (18).

MLT and Cancer

MLT exerts oncostatic activity through several biological mechanisms including antiproliferative actions, stimulation of anticancer immunity, modulation of oncogen expression, and anti-inflammatory, antioxidant and antiangiogenic effects. In addition, MLT inhibits human cancer cell growth in culture and recent clinical studies seem to confirm its anticancer properties *in vivo* (30).

Preclinical studies on MLT and cancer. The oncostatic and cytotoxic effects of MLT have been evaluated in different cell culture lines and experimental animal models, the results of which suggest that MLT possesses antiproliferative activity towards a variety of cancer cells. However, a marked variability among the findings of these studies has been possibly observed, due to methodological differences, including study parameters, culture conditions, and different MLT concentrations, doses, and duration of treatment (9).

In relation to breast cancer, most studies have been performed on chemically induced adenocarcinoma in rats and the cell tumor line MCF-7 (31). It has been shown that oncostatic actions of melatonin on hormone-dependent mammary tumors are mainly based on its antiestrogenic actions (32). MLT at a physiological concentration (1 nM) and in the presence of serum or estradiol: (a) reversibly inhibits cell proliferation; (b) increases the expression of p53 and p21/WAF1 proteins and modulates the length of the cell cycle; and (c) reduces the metastatic capacity of these cells and counteracts the stimulatory effect of estradiol on cell invasiveness; this effect is mediated, at least in part, by a melatonin-induced increase in the expression of the cell surface adhesion proteins E-cadherin and beta(1)-integrin.

The direct oncostatic effects of melatonin depend on its interaction with the tumor cell estrogen-responsive pathway. In this sense, it has been demonstrated that melatonin down-regulates the expression of ERα and inhibits the binding of the E2-ER complex to the ERE in DNA (33). Recently, it has been demonstrated that MLT inhibits estrogen production in breast adipose fibroblasts, by inhibiting transcription of the cytochrome P450, family 19, subfamily A, polypeptide 1 *(CYP19A1)* gene that encodes the key enzyme aromatase (34). Other studies have shown that melatonin signaling is modulated by antiestrogens in breast and ovarian cancer cells (35).

The inhibitory effect of MLT on colon oncogenesis has been demonstrated in different murine colon carcinoma-derived cell lines and rat models (36, 37). The decreased expression of MT1 in human colorectal cancer points to a role of MLT in this disease (38), although the oncostatic action of MLT on intestinal cancer also appears to be dependent on both MT2 and RZR/ROR receptors (39). Moreover, MLT at pharmacological concentrations has been shown to influence the expression of angiogenic and angiostatic chemokine genes in different hepatocellular carcinoma cell lines (40). Moreover, MLT suppresses the hepatocellular tumor-promoting activity of oxfenzadole in male rats through a reduction in ROS production by the activation of CYPs (41).

Studies performed *in vivo* and *in vitro* have shown that exogenous MLT exerts oncostatic effects on melanoma cells, especially through its ability to stimulate IL-2 production (23, 42), and protects cells from ultraviolet A (UVA) and B (UVB) actions (43). The radioprotective action of MLT is related to its antioxidant properties (44).

Several other investigations have assessed the signaling pathways and the expression of MLT receptors in cells lines of endometrial cancer (45), glioma (46), renal cancer (47), leukemia and hematological cancer (48, 49), prostate cancer (50), gastric adenocarcimoma (51), oral cavity tumors (52), Ewing sarcoma and bone tumors (53, 54) among others. The results of these studies have provided the basis for the potential utility of melatonin in cancer management.

Clinical studies on MLT and cancer management. Long-term disruption of circadian rhythm and reduced MLT secretion has been related to increased cancer risk (55-58). A meta-analysis of 13 observational studies reported a significant increase in the incidence of breast cancer among female airline cabin crew and female night workers, with a standardized incidence ratio of 1.44 [95% confidence interval (CI) 1.26-1.65] and 1.51 (95% CI=1.36-1.68), respectively (58). In recognition of this relationship, an expert Working Group convened by the International Agency for Cancer Research has concluded that shiftwork that involves circadian disruption is probably carcinogenic to humans (59).

Several clinical studies have been conducted in order to determine the role of MLT in the treatment of different cancer types, in conjunction with different oncological strategies on the basis of the potent antioxidant, immune-modulating, hormone-modulating, and antiproliferative actions of MLT. In order to present data supported by the best available evidence, only results from controlled randomized studies, systematic reviews and meta-analyses are reviewed here.

A recent meta-analysis of eight randomized controlled clinical trials involving 761 patients with solid tumors in which MLT was used in daily doses of 20 mg orally, as adjunct treatment concurrent with chemotherapy or radiotherapy for cancer, showed a statistically significant improvement in the complete and partial remission rate [risk ratio (RR)=1.95, 95% CI=1.49-2.54, $p<0.00001$] and 1-year survival rate (RR=1.90, 95% CI=1.28-2.83, $p=0.001$), as well as a significant decrease of radiochemotherapy-related adverse effects, such as thrombocytopenia, neurotoxicity, and fatigue (60).

These results confirm previous findings of a systematic review and meta-analysis reported by Seely *et al*. (61). This study, using data collected from 21 clinical trials, evaluated the effects of MLT in conjunction with chemotherapy, radiotherapy, supportive care, and palliative care regarding 1-year survival, complete and partial responses, stable disease, and chemotherapy-associated toxicities in 3697 patients with solid tumors (breast, colorectal, lung, renal cell, hepatocellular carcinoma, glioblastomas, and others types of cancers), most of them with advanced or metastatic disease. In the majority of trials, MLT was given at a dose of 20 mg. The pooled RR for 1-year mortality was 0.63 (95% CI=0.53-0.74, $p<0.001$). Complete response (RR=2.33, 95% CI=1.29-4.20), partial response (RR=1.90, 95% CI=1.43-2.51), and stable disease (RR=1.51, 95% CI=1.08-2.12) were all significantly improved by the addition of MLT. Moreover, MLT was found to significantly reduce occurrences of alopecia, anemia, asthenia, and thrombocytopenia.

In addition, in a systematic review of randomized controlled trials, followed by a meta-analysis, carried out by Mills *et al*. (62) in 2005, which included 10 trials published

between 1992 and 2003, with 643 patients with solid tumor cancers, MLT reduced the risk of death at one year (RR=0.66, 95% CI=0.59-0.73). It is noteworthy that in these studies, effects were consistent with MLT dose and type of cancer.

Several randomized trials have been conducted in patients with breast cancer, colorectal cancer, and lung cancer. It has been shown that MLT may stimulate ER expression and enhance the effects of tamoxifen. In a randomized study of tamoxifen *vs*. tamoxifen plus MLT (20 mg/day orally in the evening) in 40 post-menopausal patients with ER-negative metastatic breast cancer, no complete response was documented (63). However, the partial response rate was significantly higher in patients treated with the combination of tamoxifen and MLT than in those who received tamoxifen alone (36.5% *vs*. 9.5%, $p<0.05$) as was 1-year survival (63.2% *vs*. 23.8%, $p<0.01$). No MLT-related toxicity was observed; on the contrary, most patients receiving MLT experienced a relief of anxiety and of depression. This preliminary study suggests that the association of MLT may also make tamoxifen effective for patients with an ER-negative metastatic breast cancer.

In patients with advanced colorectal cancer who progressed in response to 5-fluorouracil (5-FU) plus folates, the administration of IL-2 plus MLT was evaluated in 50 patients the survival time (64). Patients were randomized to receive supportive care alone or low-dose subcutaneous IL-2 (3 million IU/day for 6 days/week for 4 weeks) plus MLT (40 mg/day orally). No spontaneous tumor regression occurred in patients receiving supportive care alone. A partial response was achieved in 3/25 patients treated with immunotherapy. The percentage of survival at one year, was significantly higher for patients treated with immunotherapy than for those treated with supportive care alone (36% *vs*. 12%, $p<0.05$). This study suggests that low-dose subcutaneous IL-2 plus MLT may be effective as a second-line therapy to induce tumor regression and to increase survival at one year in patients with metastatic colorectal cancer patients, progressing under 5-FU and folates. In another study of 30 patients with metastatic colorectal cancer progressing after at least one previous chemotherapeutic line containing 5-FU, weekly treatment with low-dose irinotecan plus MTL (20 mg/day), as compared with irinotecan alone, was associated with a higher percentage of patients with disease control (partial remission plus stable disease, 85.7% *vs*. 43.7%, $p<0.05$) (65). This preliminary study shows that the efficacy of irinotecan in pre-treated metastatic colorectal cancer may be enhanced by a concomitant daily administration of MLT.

A number of randomized trials have been conducted in patients with non-small cell lung cancer (NSCLC). In NSCLC patients resistant to first-line chemotherapy containing cisplatin, the administration of 10 mg/day of MLT (n=31) *vs*. supportive care alone (n=32) was associated with significantly higher percentages of disease stabilization and 1-year survival. Patients treated with MLT also showed a significant improvement in performance status (66). However, in patients with advanced lung cancer, treatment with MLT (40 mg/day) *vs*. placebo for 21 consecutive days, starting two days before chemotherapy with carboplatin and etoposide, did not protect against the myelotoxic effects of these cytotoxic drugs (67). In another randomized study of 60 patients with locally advanced or metastatic NSCLC, immunotherapy with low-dose IL-2 plus MLT (40 mg/day starting seven days before IL-2) *vs*. chemotherapy with cisplatin and etoposide, was associated with a significantly higher mean progression-free period and a higher percentage of survival at one year compared to chemotherapy (68). Toxicity was also substantially lower in immunotherapy-treated patients than in those treated with chemotherapy.

In a study of 70 patients in poor clinical condition with advanced NSCLC who were randomized to received first-line chemotherapy with cisplatin and etoposide or chemotherapy plus MLT (20 mg/day), higher tumor responses (32.3% *vs*. 17.1%) and percentage of 1-year survival (44.1% *vs*. 19.4%, $p<0.05$) were obtained for patients treated with MLT plus chemotherapy than for those treated with chemotherapy alone (69). In 100 consecutive patients who were randomized to receive chemotherapy alone (cisplatin and etoposide) or chemotherapy and MLT, both the overall tumor regression rate and the 5-year survival results, were significantly higher in patients concomitantly treated with MLT (70). In particular, no patient treated with chemotherapy alone was alive after two years, whereas 5-year survival was achieved in three out of 49 (6%) patients treated with chemotherapy and MLT. These data suggest a promising biochemotherapeutic strategy in the treatment of solid neoplasms.

Conclusion

MLT has revealed itself to be an ubiquitously distributed and functionally diverse molecule. Its membrane receptors are found in various organs and cell types. MLT is regarded as a chronobiotic and also as a chronobiological regulator molecule. In recent years, many investigators have implicated the pineal gland and melatonin in the processes of both aging and age-related diseases. The almost ubiquitous expression of nuclear receptors of the ROR family and other binding sites, indicates a corresponding potential for additional systemic actions, which are however, still poorly understood. The anticancer effects of MLT have been an area of extensive research in cell lines of different cancer types and in animal models. Based on evidence derived from the study of the antiproliferative, antioxidative, and immunostimulatory mechanisms of action of MLT and from clinical trials in which MLT was administered in conjunction with chemoradiotherapy and supportive care, it may be concluded

that MLT could indeed be considered a physiological anticancer substance. Further well-controlled trials with a large number of patients and longer follow-up periods should, however, be performed in order to find the link between its observed effects and the underlying mechanisms of action and to better define the significance of MLT as a therapeutic oncostatic agent.

References

1 Reiter P: Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocr Rev *12*: 151-180, 1991.

2 Vijayalaxmi, Thomas CR Jr., Reiter RJ and Herman TS: Melatonin: from basic research to cancer treatment clinics. J Clin Oncol *20*: 2575-2601, 2002.

3 Pandi-Perumal SR, Zisapel N, Srinivasan V and Cardinali DP: Melatonin and sleep in aging population. Exp Gerontol *40*: 911-925, 2005.

4 Tan DX, Manchester LC, Hardeland R, Lopez-Burillo S, Mayo JC, Sainz RM and Reiter RJ: Melatonin: a hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. J Pineal Res *34*: 75-78, 2003.

5 Hardeland R, Tan DX and Reiter RJ: Kynuramines, metabolites of melatonin and other indoles: the resurrection of an almost forgotten class of biogenic amines. J Pineal Res *47*: 109-126, 2009.

6 Luchetti F, Canonico B, Betti M, Arcangeletti M, Pilolli F, Piroddi M, Canesi L, Papa S and Galli F: Melatonin signaling and cell protection function. FASEB J *24*: 3603-3624, 2010.

7 Srinivasan V, Spence DW, Pandi-Perumal SR, Trakht I and Cardinali DP: Therapeutic actions of melatonin in cancer: possible mechanisms. Integr Cancer Ther *7*: 189-203, 2008.

8 Dubocovich ML and Markowska M: Functional MT1 and MT2 melatonin receptors in mammals. Endocrine 27: 101-110, 2005.

9 Jung B and Ahmad N: Melatonin in cancer management: progress and promise. Cancer Res *66*: 9789-9793, 2006.

10 Dubocovich ML, Cardinali DP, Guardiola-Lemaitre RM, Hagan RM, Krause DN, Sugden D *et al*: Melatonin receptiors. *In*: Girdlestone D (ed.). The IUPHAR Compendium of Receptor Characterization and Classification. IUPHAR Media: London, pp. 187-193, 1998.

11 Reppert SM: Melatonin receptors: molecular biology of a new family of G protein-coupled receptors. J Biol Rhythms *12*: 528-531, 1997.

12 Ivanova EA, Bechtold DA, Dupré SM, Brennand J, Barrett P, Luckman SM and Loudon AS: Altered metabolism in the melatonin-related receptor (GPR50) knockout mouse. Am J Physiol Endocrinol Metab *294*: E176-E182, 2008.

13 Sidibe A, Mullier A, Chen P, Baroncini M, Boutin JA, Delagrange P, Prevot V and Jockers R: Expression of the orphan GPR50 protein in rodent and human dorsomedial hypothalamus, tanycytes and median eminence. J Pineal Res *48*: 263-269, 2010.

14 Civelli O, Saito Y, Wang Z, Nothacker HP and Reinscheid RK. Orphan GPCRs and their ligands. Pharmacol Ther *110*: 525-532, 2006.

15 Levoye A, Dam J, Ayoub MA, Guillaume JL, Couturier C, Delagrange P and Jockers R: The orphan GPR50 receptor specifically inhibits MT1 melatonin receptor function through heterodimerization. EMBO J *25*: 3012-3023, 2006.

16 Tan DX, Manchester LC, Terron MP, Flores LJ, Tamura H and Reiter RJ: Melatonin as a naturally occurring co-substrate of quinone reductase-2, the putative MT3 melatonin membrane receptor: hypothesis and significance. J Pineal Res *43*: 317-320, 2007.

17 Mailliet F, Ferry G, Vella F, Berger S, Cogé F, Chomarat P, Mallet C, Guénin SP, Guillaumet G, Viaud-Massuard MC, Yous S, Delagrange P and Boutin JA: Characterization of the melatoninergic MT3 binding site on the NRH:quinone oxidoreductase 2 enzyme. Biochem Pharmacol *71*: 74-88, 2005.

18 Mediavilla MD, Sánchez-Barceló EJ, Tan DX, Manchester L and Reiter RJ: Basic mechanisms envolved in the anti-cancer effects of melatonin. Curr Med Chem *36*: 4462-4480, 2010.

19 Ravindra T, Lakshmi NK and Ahuja YR: Melatonin in pathogenesis and therapy of cancer. Indian J Med Sci *60*: 523-535, 2006.

20 Jung-Hynes B, Reiter RJ and Ahmad N: Sirtuins, melatonin and circadian rhythms: building a bridge between aging and cancer. J Pineal Res *48*: 9-19, 2010.

21 Benítez-King G, Soto-Vega E and Ramírez-Rodriguez G: Melatonin modulates microfilament phenotypes in epithelial cells: implications for adhesion and inhibition of cancer cell migration. Histol Histopathol *24*: 789-799, 2009.

22 Cos S, González A, Martínez-Campa C, Mediavilla MD, Alonso-González C and Sánchez-Barceló EJ: Estrogen-signaling pathway: a link between breast cancer and melatonin oncostatic actions. Cancer Detect Prev *30*: 118-128, 2006.

23 Srinivasan V, Pandi-Perumal SR, Brzezinski A, Bhatnagar KP and Cardinali DP: Melatonin, immune function and cancer. Recent Pat Endocr Metab Immune Drug Discov *5*: 109-23, 2011.

24 Molis TM, Spriggs LL and Hill SM: Modulation of estrogen receptor mRNA expression by melatonin in MCF-7 human breast cancer cells. Mol Endocrinol *8*: 1681-1690, 1994.

25 Rato AG, Pedrero JG, Martinez MA, del Rio B, Lazo PS and Ramos S: Melatonin blocks the activation of estrogen receptor for DNA binding. FASEB J *13*: 857-868, 1999.

26 Sainz RM, Mayo JC, Rodriguez C, Tan DX, Lopez-Burillo S and Reiter RJ: Melatonin and cell death: differential actions on apoptosis in normal and cancer cells. Cell Mol Life Sci *60*: 1407-1426, 2003.

27 Leon-Blanco MM, Guerrero JM, Reiter RJ, Calvo JR and Pozo D: Melatonin inhibits telomerase activity in the MCF-7 tumor cell line both *in vivo* and *in vitro*. J Pineal Res *35*: 204-211, 2003.

28 Park SY, Jang WJ, Yi EY, Jang JY, Jung Y, Jeong JW and Kim YJ: Melatonin suppresses tumor angiogenesis by inhibiting HIF-1alpha stabilization under hypoxia. J Pineal Res *48*: 178-184, 2010.

29 Szczepanik M. Melatonin and its influence on immune system. J Physiol Pharmacol *58(Suppl 6)*: 115-124, 2007.

30 Mediavilla MD, Sánchez-Barceló EJ, Tan DX, Manchester L and Reiter RJ: Basic mechanisms envolved in the anti-cancer effects of melatonin. Curr Med Chem *36*: 4462-4480, 2010.

31 Cos S and Sánchez-Barceló EJ: Melatonin and mammary pathological growth. Front Neuroendocrinol *21*: 133-170, 2000.

32 Hill SM, Frasch T, Xiang S, Yuan L, Duplessis T and Mao L: Molecular mechanisms of melatonin anticancer effects. Integr Cancer Ther *8*: 337-346, 2009.

33 Sánchez-Barceló EJ, Cos S, Fernández R and Mediavilla MD: Melatonin and mammary cancer: a short review. Endocr Relat Cancer *10*: 153-159, 2003.

34 Knower KC, To SQ, Takagi K, Miki Y, Sasano H, Simpson ER and Clyne CD: Melatonin suppresses aromatase expression and activity in breast cancer associated fibroblasts. Breast Cancer Res Treat *132*: 765-771, 2012.

35 Treeck O, Haldar C and Ortmann O: Antiestrogens modulate MT1 melatonin receptor expression in breast and ovarian cancer cell lines. Oncol Rep *15*: 231-235, 2006.

36 Farriol M, Venereo Y, Orta X, Castellanos JM and Segovia-Silvestre T: In vitro effects of melatonin on cell proliferation in a colon adenocarcinoma line. J Appl Toxicol *20*: 21-24, 2000.

37 Tanaka T, Yasui Y, Tanaka M, Tanaka T, Oyama T and Rahman KM: Melatonin suppresses AOM/DSS-induced large bowel oncogenesis in rats. Chem Biol Interact *177*: 128-136, 2009.

38 Nemeth C, Humpeler S, Kallay E, Mesteri I, Svoboda M, Rögelsperger O, Klammer N, Thalhammer T and Ekmekcioglu C: Decreased expression of the melatonin receptor 1 in human colorectal carcinomas. J Biol Regul Homeost Agents *25*: 531-542, 2011.

39 Winczyk K, Pawlikowski M, Lawnicka H, Kunert-Radek J, Spadoni G, Tarzia G and Karasek M: 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 *23(Suppl 1)*: 50-54, 2002.

40 Lin ZY and Chuang WL: Pharmacologic concentrations of melatonin have diverse influence on differential expressions of angiogenic chemokine genes in different hepatocellular carcinoma cell lines. Biomed Pharmacother *64*: 659-662, 2010.

41 Nishimura J, Saegusa Y, Dewa Y, Jin M, Kawai M, Kemmochi S, Harada T, Hayashi SM, Shibutani M and Mitsumori K: Antioxidant enzymatically modified isoquercitrin or melatonin supplementation reduces oxidative stress-mediated hepatocellular tumor promotion of oxfendazole in rats. Arch Toxicol *84*: 143-153, 2010.

42 Cabrera J, Negrín G, Estévez F, Loro J, Reiter RJ and Quintana J: Melatonin decreases cell proliferation and induces melanogenesis in human melanoma SK-MEL-1 cells. J Pineal Res *49*: 45-54, 2010.

43 Izykowska I, Gebarowska E, Cegielski M, Podhorska-Okolow M, Piotrowska A, Zabel M and Dziegiel P: Effect of melatonin on melanoma cells subjected to UVA and UVB radiation in i*n vitro* studies. In Vivo *23*: 733-738, 2009.

44 Kim BC, Shon BS, Ryoo YW, Kim SP and Lee KS: Melatonin reduces X-ray irradiation-induced oxidative damages in cultured human skin fibroblasts. J Dermatol Sci *26*: 194-200, 2001.

45 Watanabe M, Kobayashi Y, Takahashi N, Kiguchi K and Ishizuka B: Expression of melatonin receptor (MT1) and interaction between melatonin and estrogen in endometrial cancer cell line. J Obstet Gynaecol Res *34*: 567-573, 2008.

46 Esposito E, Iacono A, Muià C, Crisafulli C, Mattace Raso G, Bramanti P, Meli R and Cuzzocrea S: Signal transduction pathways involved in protective effects of melatonin in C6 glioma cells. J Pineal Res *44*: 78-87, 2008.

47 Min KJ, Kim HS, Park EJ and Kwon TK: Melatonin enhances thapsigargin-induced apoptosis through reactive oxygen species-mediated upregulation of CCAAT-enhancer-binding protein homologous protein in human renal cancer cells. J Pineal Res 2012, doi: 10.1111/j.1600-079X.2012.00975.x.

48 Koh W, Jeong SJ, Lee HJ, Ryu HG, Lee EO, Ahn KS, Bae H and Kim SH: Melatonin promotes puromycin-induced apoptosis with activation of caspase-3 and 5'-adenosine monophosphate-activated kinase-alpha in human leukemia HL-60 cells. J Pineal Res *50*: 367-373, 2011.

49 Casado-Zapico S, Martín V, García-Santos G, Rodríguez-Blanco J, Sánchez-Sánchez AM, Luño E, Suárez C, García-Pedrero JM, Menendez ST, Antolín I and Rodriguez C: Regulation of the expression of death receptors and their ligands by melatonin in haematological cancer cell lines and in leukaemia cells from patients. J Pineal Res *50*: 345-355, 2011.

50 Tam CW and Shiu SY: Functional interplay between melatonin receptor-mediated antiproliferative signaling and androgen receptor signaling in human prostate epithelial cells: potential implications for therapeutic strategies against prostate cancer. J Pineal Res *51*: 297-312, 2011.

51 Zhang S, Zuo L, Gui S, Zhou Q, Wei W and Wang Y: Induction of cell differentiation and promotion of endocan gene expression in stomach cancer by melatonin. Mol Biol Res *39*: 2843-2849, 2012.

52 Cutando A, Aneiros-Fernández J, Aneiros-Cachaza J and Arias-Santiago S: Melatonin and cancer: current knowledge and its application to oral cavity tumours. J Oral Pathol Med *40*: 593-597, 2011.

53 Casado-Zapico S, Rodriguez-Blanco J, García-Santos G, Martín V, Sánchez-Sánchez AM, Antolín I and Rodriguez C: Synergistic antitumor effect of melatonin with several chemotherapeutic drugs on human Ewing sarcoma cancer cells: potentiation of the extrinsic apoptotic pathway. J Pineal Res *48*: 72-80, 2010.

54 Toma CD, Svoboda M, Arrich F, Ekmekcioglu C, Assadian O and Thalhammer T: Expression of the melatonin receptor (MT) 1 in benign and malignant human bone tumors. J Pineal Res *43*: 206-213, 2007.

55 Reiter RJ, Tan DX, Korkmaz A, Erren TC, Piekarski C, Tamura H and Manchester LC: Light at night, chronodisruption, melatonin suppression, and cancer risk: a review. Crit Rev Oncog *13*: 303-328, 2007.

56 Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I and Colditz GA: Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. J Natl Cancer Inst *93*: 1563-1568, 2001.

57 Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, Fuchs CS and Colditz GA: Night-shift work and risk of colorectal cancer in the nurses' health study. J Natl Cancer Inst *95*: 825-828, 2003.

58 Megdal SP, Kroenke CH, Laden F, Pukkala E and Schernhammer ES: Night work and breast cancer risk: a systematic review and meta-analysis. Eur J Cancer *41*: 2023-2032, 2005.

59 International Agency for Research on Cancer. IARC Monographs Programme finds cancer hazards associated with shiftwork, painting and firefighting. Press release no. 180, 5 December 2007. http://www.iarc.fr/en/media-centre/pr/2007/pr180.html

60 Wang YM, Jin BZ, Ai F, Duan CH, Lu YZ, Dong TF and Fu QL: The efficacy and safety of melatonin in concurrent chemotherapy or radiotherapy for solid tumors: a meta-analysis of randomized controlled trials. Cancer Chemother Pharmacol 2012, doi: 10.1007/s00280-012-1828-8.

61 Seely D, Wu P, Fritz H, Kennedy DA, Tsui T, Seely AJ and Mills E: Melatonin as adjuvant cancer care with and without chemotherapy: a systematic review and meta-analysis of randomized trials. Integr Cancer Ther 2011, doi: 10.1177/1534735411425484.

62 Mills E, Wu P, Seely D and Guyatt G: Melatonin in the treatment of cancer: a systematic review of randomized controlled trials and meta-analysis. J Pineal Res *39*: 360-366, 2005.

63 Lissoni P, Ardizzoia A, Barni S, Paolorossi F, Tancini G, Meregalli S, Esposti D, Zubelewicz B and Braczowski R: A randomized study of tamoxifen alone versus tamoxifen plus melatonin in estrogen receptor-negative heavily pretreated metastatic breast-cancer patients. Oncol Rep *2*: 871-873, 1995.

64 Barni S, Lissoni P, Cazzaniga M, Ardizzoia A, Meregalli S, Fossati V, Fumagalli L, Brivio F and Tancini G: A randomized study of low-dose subcutaneous interleukin-2 plus melatonin versus supportive care alone in metastatic colorectal cancer patients progressing under 5-fluorouracil and folates. Oncology *52*: 243-245, 1995.

65 Cerea G, Vaghi M, Ardizzoia A, Villa S, Bucovec R, Mengo S, Gardani G, Tancini G and Lissoni P: Biomodulation of cancer chemotherapy for metastatic colorectal cancer: a randomized study of weekly low-dose irinotecan alone versus irinotecan plus the oncostatic pineal hormone melatonin in metastatic colorectal cancer patients progressing on 5-fluorouracil-containing combinations. Anticancer Res *23*: 1951-1954, 2003.

66 Lissoni P, Barni S, Ardizzoia A, Paolorossi F, Crispino S, Tancini G, Tisi E, Archili C, De Toma D, Pipino G, Conti A and Maestroni GJM: Randomized study with the pineal hormone melatonin versus supportive care alone in advanced nonsmall cell lung cancer resistant to a first-line chemotherapy containing cisplatin. Oncology *49*: 336-339, 1992.

67 Ghielmini M, Pagani O, de Jong J, Pampallona S, Conti A, Maestroni G, Sessa C and Cavalli F: Double-blind randomized study on the myeloprotective effect of melatonin in combination with carboplatin and etoposide in advanced lung cancer. Br J Cancer *80*: 1058-1061, 1999.

68 Lissoni P, Meregalli S, Fossati V, Paolorossi F, Barni S, Tancini G and Frigerio F: A randomized study of immunotherapy with low-dose subcutaneous interleukin-2 plus melatonin vs chemotherapy with cisplatin and etoposide as first-line therapy for advanced non-small cell lung cancer. Tumori *80*: 464-467, 1994.

69 Lissoni P, Paolorossi F, Ardizzoia A, Barni S, Chilelli M, Mancuso M, Tancini G, Conti A and Maestroni GJ: A randomized study of chemotherapy with cisplatin plus etoposide versus chemoendocrine therapy with cisplatin, etoposide and the pineal hormone melatonin as a first-line treatment of advanced non-small cell lung cancer patients in a poor clinical state. J Pineal Res *23*: 15-19, 1997.

70 Lissoni P, Chilelli M, Villa S, Cerizza L and Tancini G: Five years survival in metastatic non-small cell lung cancer patients treated with chemotherapy alone or chemotherapy and melatonin: a randomized trial. J Pineal Res *35*: 12-15, 2003.

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