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Melatonin and mammary cancer: a short review

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Abstract

Melatonin is an indolic hormone produced mainly by the pineal gland. The former hypothesis of its possible role in mammary cancer development was based on the evidence that melatonin down-regulates some of the pituitary and gonadal hormones that control mammary gland development and which are also responsible for the growth of hormone-dependent mammary tumors. Furthermore, melatonin could act directly on tumoral cells, as a naturally occurring antiestrogen, thereby influencing their proliferative rate. The first reports revealed a low plasmatic melatonin concentration in women with estrogen receptor (ER)-positive breast tumors. However, later studies on the possible role of melatonin on human breast cancer have been scarce and mostly of an epidemiological type. These studies described a low incidence of breast tumors in blind women as well as an inverse relationship between breast cancer incidence and the degree of visual impairment. Since light inhibits melatonin secretion, the relative increase in the melatonin circulating levels in women with a decreased light input could be interpreted as proof of the protective role of melatonin on mammary carcinogenesis. From *in vivo* studies on animal models of chemically induced mammary tumorigenesis, the general conclusion is that experimental manipulations activating the pineal gland or the administration of melatonin lengthens the latency and reduces the incidence and growth rate of mammary tumors, while pinealectomy usually has the opposite effects. Melatonin also reduces the incidence of spontaneous mammary tumors in different kinds of transgenic mice (*c-neu* and *N-ras*) and mice from strains with a high tumoral incidence.

In vitro experiments, carried out with the ER-positive MCF-7 human breast cancer cells, demonstrated that melatonin, at a physiological concentration (1 nM) and in the presence of serum or estradiol: (a) inhibits, in a reversible way, cell proliferation, (b) increases the expression of p53 and p21WAF1 proteins and modulates the length of the cell cycle, and (c) reduces the metastasic 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 β_1-integrin.

The direct oncostatic effects of melatonin depends 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 estradiol–ER complex to the estrogen response element (ERE) in the DNA. The characteristics of melatonin's oncostatic actions, comprising different aspects of tumor biology as well as the physiological doses at which the effect is accomplished, give special value to these findings and encourage clinical studies on the possible therapeutic value of melatonin on breast cancer.

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Introduction

Melatonin is an indolic hormone mainly secreted by the pineal gland. One of the most striking characteristics of this hormone is that it is secreted only during the night, or more exactly, in darkness. Consequently, the melatonin concentration in plasma is low during the day (in light) and reaches a peak value of about 1 nM during the night (in darkness). The nocturnal secretion of melatonin is very sensitive to the inhibitory effects of light, and the exposure to light at night, even if it is a short-time exposure to light of low intensity, can cause the nocturnal melatonin peak to become either decreased or fully suppressed (Brainard *et al.* 1997).

Among the proposed actions of melatonin (Reiter 1980, Brzezinski 1997), those supporting the hypothesis of a possible oncostatic role of melatonin on hormone-dependent tumors are: (a) the down-regulation of the hormones of the neuroendocrine reproductive axis, leading to a decrease in the circulating levels of gonadal steroids, and (b) the ability of melatonin to counteract the effects of estrogens at the level of the estrogen targets, that is to say, to behave as a naturally occurring antiestrogen. Both the enhancement of immune function induced by melatonin (Maestroni 1993) as well as the antioxidant properties of this indolamine (Reiter *et al.* 1995) have also been considered among those mechanisms involved in its oncostatic actions. However, what we are going to analyze in this article are those melatonin antitumoral effects dependent on its interaction with the hormones of the reproductive axis, focusing our attention on the role of melatonin on hormone-dependent mammary cancer.

The hypothesis on the role of the pineal gland and melatonin in human breast cancer

In 1978 an article published in *The Lancet* (Cohen *et al.* 1978) introduced, for the first time, the theory of the possible role of the pineal gland on the etiology of breast cancer. These authors suggested that a decrease in pineal function, whatever its cause, and the consequent reduction in melatonin secretion, could induce a state of relative hyperestrogenism, and the early and prolonged exposure of the breast tissue to the estrogens could be involved in the etiology of the breast tumor. A few years later, Tamarkin *et al.* (1982), in an article in *Science*, described a relationship between plasma melatonin concentration and breast cancer. Women with estrogen receptor (ER)-positive breast adenocarcinomas had nocturnal plasmatic concentrations of melatonin significantly lower than healthy women or women suffering ER-negative breast tumors. Whether these changes in melatonin secretion were the origin or the consequence of the tumoral process was not, however, clarified.

From that promising article until now, the data supporting a possible role of melatonin on human breast cancer have, unfortunately, been very scarce and most of them consist of indirect evidence resulting from epidemiological studies. These studies suggest a relationship between pineal function and the risk of breast cancer, based on the low incidence of breast cancer among blind women (Coleman & Reiter 1992, Feychting *et al.* 1998, Kliukiene *et al.* 2001) as well as on the inverse association between breast cancer incidence and degree of visual impairment (Verkasalo *et al.* 1999). In these cases, the total or partial suppression of the light input could mediate an increase in melatonin circulating levels that could explain the low incidence of tumors. On the other hand, the high incidence of breast cancer among women exposed to light during the night (such as shift workers) (Kheifets & Matkin 1999) or exposed to low frequency electromagnetic fields (Brainard *et al.* 1999, Caplan *et al.* 2000) could be explained by the decreased melatonin synthesis under these environmental conditions.

Despite this apparent lack of interest in the possible oncostatic role of melatonin in human breast cancer, we consider that evidence from basic studies carried out on animal models, as well as *in vitro* with tumoral cell lines, is so consistent as to consider it interesting to work in the area of this hypothesis.

Evidence from *in vivo* studies on animal models

Most *in vivo* studies have used, as an animal model, the chemically induced (7,12-dimethylbenz[a]anthracene or N-nitrosomethylurea) mammary cancer in rats (Cos & Sánchez-Barceló 2000*a*,*b*). In spite of the great diversity of experimental approaches undertaken by the different groups involved in this research, what they all have in common is that they are based on a comparison between the effects of the carcinogen in animals with something equivalent to an increased pineal function and those effects on animals with something equivalent to decreased or suppressed pineal function. An increased pineal function can be achieved by subjecting animals to experimental manipulations known as enhancers of melatonin's antigonadotropic actions (anosmia, underfeeding, cold exposure, etc.), by exposing animals to short photoperiods which increase melatonin levels, or by directly administering melatonin. Pineal function can be suppressed by surgical pinealectomy or exposure to continuous light, or decreased by exposing the animals to very long photoperiods. From these kinds of experiments, the general conclusions are that animals with enhanced pineal function or those treated with melatonin, in contrast to pinealectomized animals or to animals with decreased melatonin levels, have: (a) increased tumoral latency (the time elapsing between the administration of the carcinogen and the appearance of palpable mammary tumors), (b) a significantly lower tumoral incidence (% of animals developing tumors), (c) a reduction in the number and size of the tumors, (d) a greater

incidence of fibroadenomas than of adenocarcinomas, (e) a lower rate of tumoral growth, and (f) a more frequent incidence of spontaneous tumor regression (Cos & Sánchez-Barceló 2000*a*,*b*).

Another kind of *in vivo* study was carried out in murine strains such as C3H/Jax mice with a high incidence of spontaneous mammary tumors. In these animals, prolonged oral melatonin treatment significantly reduces the development of mammary tumors (Subramanian & Kothari 1991). Finally, other *in vivo* experiments have been carried out in mice overexpressing genes involved in mammary carcinogenesis. Thus, transgenic mice overexpressing the N-*ras* protooncogene under the transcriptional control of the MMTV-mouse mammary tumor virus – long terminal repeat (LTR) develop hyperplasic alveolar nodules (premalignant lesions) as well as mammary adenocarcinomas. The treatment of these transgenic mice with melatonin significantly reduces the incidence of these mammary lesions, the expression of N-ras protein in focal hyperplasic lesions, and the incidence of adenocarcinomas (Mediavilla *et al*. 1997). In transgenic mice expressing the *c-neu* breast cancer oncogene under the control of an MMTV promoter, melatonin delayed the appearance of palpable tumors and the growth of the tumors (Rao *et al*. 2000).

These oncostatic effects of melatonin *in vivo* could be explained by the down-regulatory effects of this indolamine on the neuroendocrine reproductive axis (Reiter 1980, Brzezinski 1997), and the consequent reduction of hormones such as prolactin and to a large extent estradiol, which are responsible for the normal and pathological growth of the mammary epithelium.

Evidence from *in vitro* studies on human breast cancer cells

The direct antiestrogenic effects of melatonin on breast cancer cells were evidenced from *in vitro* studies (Cos & Sánchez-Barceló 2000*a*,*c*). Most of them were carried out on MCF-7 human breast cancer cells. The characteristics of these cells are well known and comprise the expression of ERα and ERβ as well as the wild-type of the tumor suppresor protein p53. Recently, the expression of MT1 melatonin receptors in these cells has been demonstrated (Ram *et al*. 1998, Yuan *et al*. 2002). In these cells melatonin inhibits, in a reversible way, cell proliferation. These antiproliferative effects have some important characteristics such as: (a) they are dependent on the presence of complete serum or stripped serum plus estradiol in the culture media, (b) they are dose-dependent and only melatonin concentrations close to 1 nM (the nocturnal concentration in plasma of most mammals) are effective for decreasing cell proliferation, whereas supra- or sub-physiological ones lack these antiproliferative effects, (c) the inhibitory effect is not shared with melatonin precursors, metabolites or other pineal methoxyindoles, (d) they are dependent on the rate of cell growth, where the greater the rate of cell proliferation is the higher the level of the melatonin antiproliferative actions, and (e) they are dependent on the pattern (continuous or pulsated) of the exposure to melatonin in the culture media, thus the highest antiproliferative effects are obtained when the concentration of melatonin in culture media is changed every 12 h between 10 pM and 1 nM, thus mimicking the physiological day/night oscillation of melatonin in the plasma of most mammals (Cos & Sánchez-Barceló 1994, 2000*a*,*c*).

The antiproliferative effects of melatonin are related to its modulatory effects on the cell cycle. Melatonin, in the presence of normal serum or estradiol, has been shown to retard or block the progression of cells from G0-G1 into S phase; thus, when cells are incubated with 1 nM melatonin, an accumulation of cells in G0-G1 together with a decrease in the population of cells in S phase can be observed (Cos *et al*. 1991, García-Rato *et al*. 1999). Melatonin also increases the length of the MCF-7 cell cycle from 20.36 ± 0.52 to 23.48 ± 0.03 h (Cos *et al*. 1996). These modulatory effects of melatonin on the cell cycle could be explained by the effects of this indolamine on the expression of some of the proteins involved in the control of the G1–S transition. Thus, it has been demonstrated that melatonin, at nanomolar concentrations, increases the expression of p53 and p21WAF1 (Mediavilla *et al*. 1999). However, despite the increase in p53, melatonin does not seem to induce apoptosis in these cells (Cos *et al*. 2002). Melatonin not only reduces MCF-7 cell proliferation but also their metastasic capacity. In an *in vitro* study, we demonstrated that 1 nM melatonin reduced the invasiveness of tumoral cells measured in Falcon invasion chambers and was also able to block estradiol-induced invasion (Cos *et al*. 1998). These effects are mediated, at least in part, by a melatonin-induced increase in the expression of two cell surface adhesion proteins, E-cadherin and β_1-integrin (Cos *et al*. 1998), as well as by an increased gap junctional intercellular communication between adjacent epithelial cells also induced by melatonin (Cos & Fernández 2000).

Mechanisms involved in the effects of melatonin on MCF-7 cells

There is general agreement that melatonin's oncostatic effects on these cells are dependent on its interaction with the tumour cell estrogen-responsive pathway. The data which uphold this hypothesis are: (a) melatonin inhibits proliferation only in those cells expressing ERα (Cos & Sánchez-Barceló 2000*a*), (b) melatonin blocks the mitogenic effects of estradiol as well as counteracts the estradiol-induced invasiveness of MCF-7 cells (Cos *et al*. 1998), (c) melatonin potentiates the sensitivity of MCF-7 cells to antiestrogens such as tamoxifen (Wilson *et al*. 1992), (d) the transfection of MT1 melatonin receptors to MCF-7 cells (ERα positive)

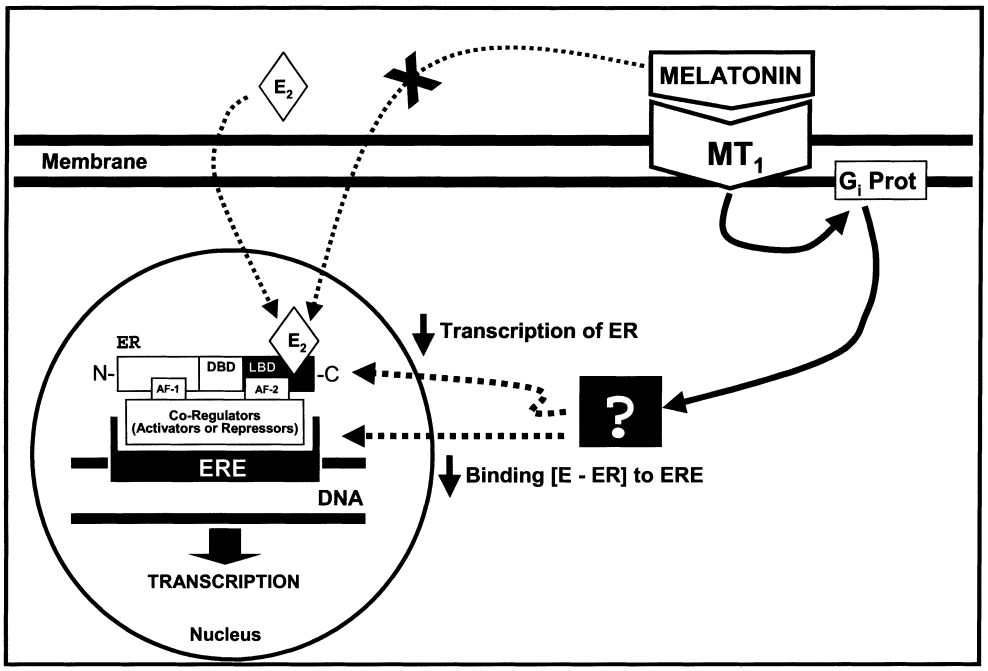

Figure 1 Mechanism of melatonin antiestrogenic effects on MCF-7 cells. Melatonin does not bind to the estrogen receptor (ER) nor interfere with the binding of estradiol (E_2, E) to the ER. Melatonin decreases the expression of ER and inhibits the binding of the E–ER complex to the estrogen response element (ERE) in the DNA (see references in text). DBD, DNA binding domain; LBD, ligand binding domain; AF-1, transcriptional activation function 1; AF-2, transcriptional activation function 2; MT_1, melatonin receptor.

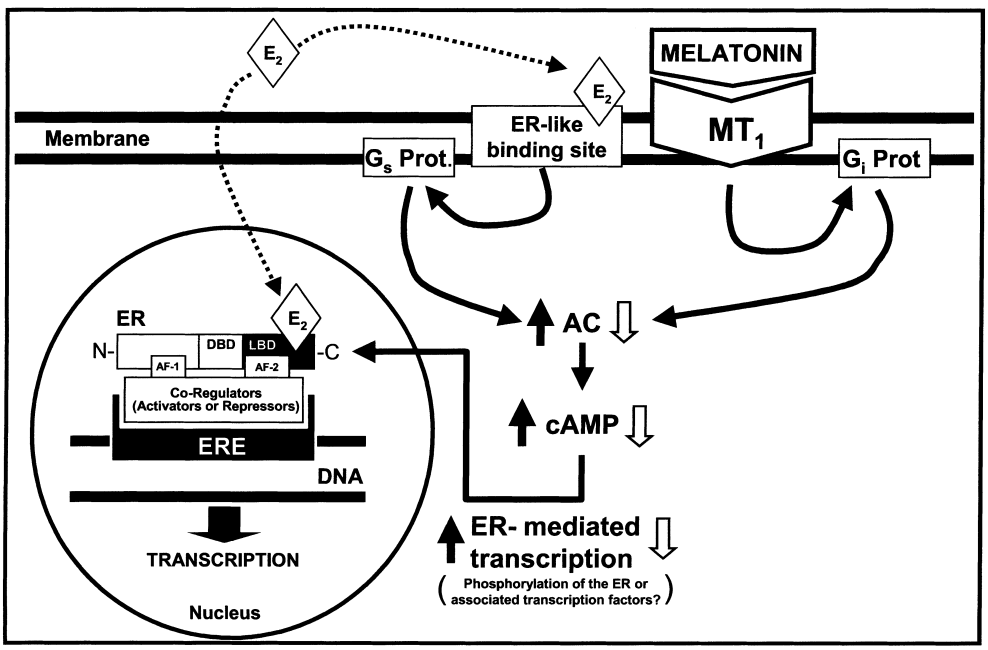

Figure 2 cAMP as the link between the estrogen-signaling pathway and melatonin. Estrogens, through their binding to high-affinity membrane binders, activate adenylate cyclase (AC) increasing the concentration of cAMP in ER-responsive breast cancer cells; cAMP synergises with ER-occupied receptors enhancing ER-mediated transcription. Melatonin, after its binding to melatonin membrane receptors, inhibits AC and decreases cAMP, thus counteracting the effects of the estrogens (see references in text).

or MDA-MB-231 cells (ERα negative) significantly enhances the growth-suppression effects of melatonin only in MCF-7 cells, that is to say, in those also expressing ER (Yuan *et al*. 2002), and (e) melatonin inhibits the expression of estrogen-regulated genes such as pS2 or cathepsine (Molis *et al*. 1995).

How melatonin interacts with the estrogen-signaling pathway is an open question. Evidence indicates that melatonin does not bind to the ER nor interfere with the binding of estradiol to its receptor (Molis *et al*. 1994, García-Rato *et al*. 1999) (Fig. 1). This is a point that differentiates melatonin from the classic antiestrogens such as tamoxifen, ICI 164,384 and its derivatives. The effects of melatonin consist of a decrease in the expression of ERα as well as the inhibition of the binding of the estradiol–ER complex to the estrogen response element (ERE) on DNA (Lawson *et al*. 1992, Molis *et al*. 1994, García-Rato *et al*. 1999), and these effects depend on its binding to a high affinity membrane-bound receptor coupled to Gi proteins (Jones *et al*. 2000, Ram *et al*. 2002, Yuan *et al*. 2002).

The possible link between the signaling pathways of estrogens and melatonin has not been elucidated. Cyclic AMP has recently been suggested as one of these possible links between both signaling pathways (Kiefer *et al*. 2002) (Fig. 2). Cyclic AMP and other protein kinase activators have been documented to synergize with steroid hormone-occupied receptors, leading to enhanced ER-mediated transcription, possibly by a mechanism involving phosphorylation of the ER or associated transcription factors (Arónica *et al*. 1994). Estrogens activate adenylate cyclase (AC), markedly increasing the concentration of cAMP in ER-responsive breast cancer cells in culture in a manner that does not require new mRNA or protein synthesis, and is mediated by a high-affinity hormone binder (possibly ER). Melatonin, after its binding to melatonin membrane receptors, inhibits the AC and decreases cAMP, thus counteracting the effects of estrogens.

Another possible link between melatonin and the estrogen signaling pathway could be calmodulin (CaM). Since the demonstration of the interaction of CaM with the ER (Castoria *et al*. 1988), several reports have indicated that antiestrogens and anti-CaM drugs inhibit MCF-7 cell proliferation by stopping the cell cycle at the G1 phase (Musgrove *et al*. 1989). Recently, it has been specified that ERα but not ERβ has a CaM binding site and interacts with CaM (García-Pedrero *et al*. 2002). The binding of CaM to ERα stimulates the phosphorylation of the receptor, thus facilitating the binding of the estrogen as well as the binding of the estradiol–ER complex to the ERE (Bouhoute & Leclercq 1995, García-Pedrero *et al*. 2002). Melatonin modulates the Ca^{++}/CaM signaling pathway either by changing the intracellular Ca^{++} concentration via activation of its G-protein coupled membrane receptors, or through a direct interaction with CaM (Benitez-King *et al*. 1993, 1996). Melatonin's

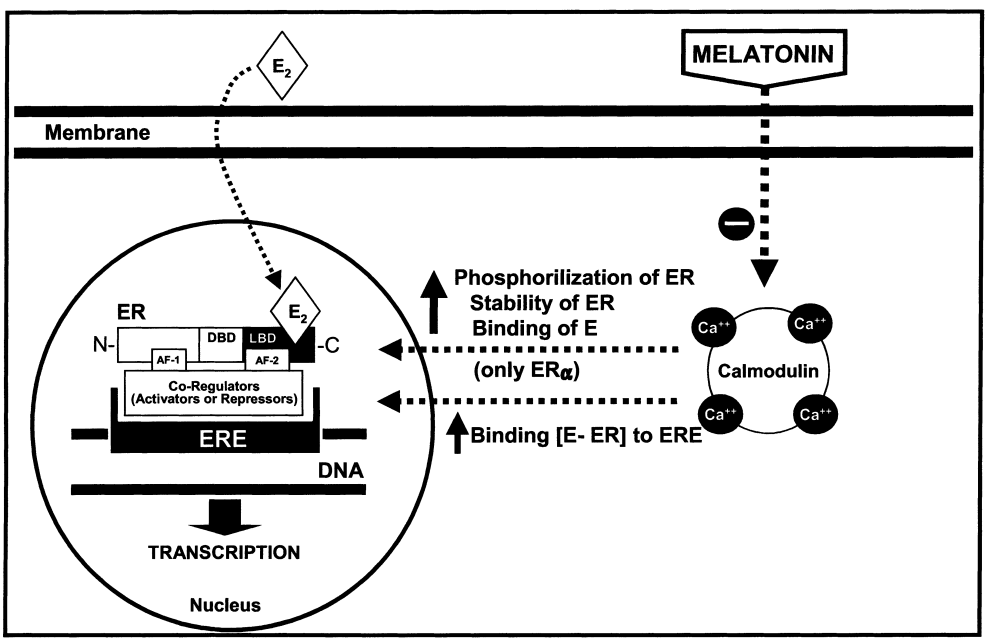

Figure 3 Calmodulin as a possible link between melatonin and the estrogen-signaling pathway. Calmodulin binds to ERα (not to ERβ) and stimulates the phosphorylation of the ER thus facilitating the binding of estradiol (E) as well as the binding of the E–ER complex to the ERE; melatonin binds and inactivates calmodulin thus counteracting the effects of estrogens (see references in text).

binding and inactivation of CaM could be the mechanism by which it exerts its antiestrogenic effects (García-Rato *et al*. 1999, Dai *et al*. 2002) (Fig. 3).

Conclusions

(1) Melatonin, through its antigonadotropic and antiestrogenic actions, behaves as an antitumoral agent on hormone-dependent mammary tumors. (2) These oncostatic properties of melatonin have consistently been demonstrated on *in vivo* models of chemically induced rat mammary tumors as well as *in vitro* on MCF-7 human breast cancer cells. (3) The possible role of melatonin on human breast tumors is only supported by indirect data. (4) The characteristics of melatonin's oncostatic actions, comprising different aspects of tumor biology such as initiation, proliferation, and metastasis as well as the physiological doses at which the effect is accomplished, give special value to these findings and encourage clinical studies on the possible therapeutic value of melatonin in breast cancer.

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