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REVIEW ARTICLE

The light-dark regimen and cancer development

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Abstract

The role of the modulation of the pineal gland function in development of cancer is discussed in the review. An inhibition of the pineal function with pinealectomy or with the exposure to the constant light regimen stimulates mammary carcinogenesis, whereas the light deprivation inhibits the carcinogenesis. Epidemiological observations on increased risk of breast cancer in night shift workers, flight attendants, radio and telegraph operators and on decreased risk in blind women are in accordance with the results of experiments in rodents. Treatment with pineal indole hormone melatonin inhibits carcinogenesis in pinealectomized rats or animals kept at the standard light/dark regimen (LD) or at the constant illumination (LL) regimen.

Introduction

According to the International Agency for Research on Cancer report [1], breast cancer constituted a huge disease burden in developed countries in the year 2000. It is the most common cancer in women with an estimated 999,000 new cases of breast cancer each year (about 22% of cancers in women) resulting in some 375,000 deaths. More than half of all cases are registered in industrialized countries: about 335,000 in Europe and 195,000 in North America. The disease is not yet as common among women in developing countries although proliferation is increasing. Risk of breast cancer incidence had been associated with higher socio-economic status such as income, education, housing, etc. as they were related to such health factors as age at menstruation and menopause, obesity, height, alcohol consumption, late age at first birth, low parity, estrogen replacement therapy, some diet habits, etc. Two conditions unique to developed countries are an increasing exposure to light-at-night and power frequency (50–60 Hz) magnetic fields.

The light-at-night is a life style factor now shown to be significant. The alternation of the day and night circadian cycle is a most important regulator of a wide variety of physiological rhythms in living organisms. Light exposure at night has been found to be related to a number of serious behavioral as well as health problems including cancer. Fruit fly experiments have shown that exposure to constant light (24 hours a day, below – LL) was followed by a shortening of their life spans [2,3]. In rodents, the light-at-night leads to disruption of the ovulatory cycle followed by hyperplastic processes in mammary gland, ovarian and uterine tumor development [4,5]. There was shown the tumor-promoting effect of exposure to the LL regimen on mammary gland carcinogenesis in mice and rats [7–10]. Prolonged light exposure suppresses the

night peak release of melatonin – the 'hormone of the night' [8,10–13]. Melatonin is a principal hormone of the pineal gland – the small neuroendocrine gland connected with the brain which mediates information on light from the retina of the eyes to the organism [11,14].

Recently, the Journal of the National Cancer Institute [15,16] published two papers reporting a significant increase in the risk of breast cancer among women who frequently did not sleep during the period of the night, about 1:30 a.m., when melatonin levels are typically at their highest. There was increased risk among women sleeping in the brightest bedrooms. Moreover, women who had worked 30 and more years on rotating night shifts had a 36% greater risk of breast cancer compared with workers who had never worked nights. Earlier, epidemiologists had shown an elevated breast cancer risk among post-menopausal radio and telegraph operators exposed to shift work as well as among flight attendants working markedly random night periods [17–20]. 'Melatonin hypothesis' suggests that reduced pineal melatonin production might increase human breast cancer risk because lower melatonin output would lead to an increase in the level of female sex hormones and would stimulate proliferation of breast tissue [10,21]. However, the exact mechanisms of the connection of melatonin inadequacy with breast cancer had not been well explored. In the the present paper we will review evidences that modulation of the pineal gland function by light/dark regimen and with pineal hormone and peptides could stop breast cancer burden. The data on effect of low-frequency electromagnetic fields (50–60 Hz) which were intensively discussed in several recent reviews [8,10,22,23], and the results of clinical trials of melatonin and pineal peptides are behind a scope of this review.

Effect of constant light regimen on spontaneous mammary carcinogenesis

Artificial increase the length of light phase of the day (by 2–4 hours) as usual followed by the increase in the duration of estral cycle and in some cases to it disturbances. If the light will be switched-on for 24 hours per day the majority of female mice and rats in a short period revealed the persistent estrus syndrome. In physiologcal circumstances, this syndrome naturaly develops at some age (in rats, as usual between 15th and 18th months) and preceeds to the anestrus [24], being the physiological equivalent of climacteric syndrome and climacteric in women. The ovary of persistent-estrus rats content follicular cysts hyperplasia theca-tissue, whereas the corpora lutea are absent [5,25]. Instead cylcic production of gonadotropins, prolactin, estrogens and progesterone characteristic for normal reproductive period of life, their acyclic production followed by hyperplastic processes in mammary gland and uterus [25,26]. The decrease of tolerance to glucose and of the sensitivity to insulin have been observed in rats with the persistent-estrus rats [27]. We have found that the exposure to the LL regimen leads to the increase in the threshold of sensitivity of the hypothalamus to the feedback inhibition by estrogens in female rats [28]. This mechanism is a key mechanism in the aging of reproductive system in female rats as well as in women [28–30].

The exposure to the constant light regimen being started before maturity leads to its acceleration and to fast (in 4–8 weeks) development of the persistent estrus syndrome. In 15 weeks after the start of the experiment follilcular cysts in the ovaries had 92% rats of the LL groups and no cases of the ovarian cysts development were observed in the control (LD) group [5]. The very similar disturbances observed in rats when the exposure to the LL regimen have been started at the age of 2 months. It is very important that the follicular cysts in the ovaries and the persistent estrus is not stopped if rats were placed then in the room with the standard LD regimen [5].

The development of hyperplastic processes and of the mastopathy was registered in 78–88% of female rats in 7 months after the start of the exposure to the LL regimen and the first cases of the mastopathy have been registered in 4.5 – 6 months after the start of the exposure to the LL [4]. The development of mammary fibroadenomas and adenocarcinomas was registered in the LL group. The cystic-adenomatous hyperplasia and fibrous polyps of endometrium developed also in the rats in the LL group. No cases of mastopathy were registered in rats of the control LD group at the 7th month after start of the observation. In all cases of finding of the mastopathy, follicular ovarian cysts were observed in the same animal as well. The level of FSH was increased and the level of of LH decreased in the pituitary of rats in the LL group as compared to the LD group.

Administration of melatonin (50 or 100 μg/rat daily) was followed by normalization of the estrus function and by the prevention of follicular ovarian cysts and mammary hyperplastic processes and mastopathy in rats kept during 8 month under the LL regimen. The preventive effect of melatonin was proportional to it dose [5].

The exposure to the LL regimen failed to change the incidence of spontaneous mammary adenocarcinoma development, the size of mammary tumors, as well as the incidence and size of lung metastases in transgenic HER-2/neu female mice. However, the number of tumors per mouse was significantly increased in the LL group as compared to the LD group. The number of mice bearing 4 and more tumors was higher in the LL group than in the LD group, whereas the number of mice bearing 1 to 3 tumors was lower in the LL group in comparison with the LD group [31].

The exposure to LL regimen accelerated development of spontaneous mammary carcinomas in female C3H-A mice but not in C3H-HeJ mice [32]. Moreover, it was observed an increase in mammary tumor latency and delay in its growth as compared to mice kept at LD regimen. The author noted that genetically-dependent retinal degeneration in C3H-HeJ mice can determine teir insensitivity to the light.

Effect of constant light regimen on chemically-induced mammary carcinogenesis

In 1965, Khaetsky [6] firstly reported the effect of exposure to constant light regimen (LL) on DMBA-induced mammary carcinogenesis in outbred rats. Female rats were kept at standard (12 hours ligh/12 hours dark) regimen (LD) or at the LL regimen during 7 weeks and then were exposed to 5 weekly intravenous injections of DMBA in a single dose 1.5 mg/rat. Mammary tumors developed in 40% LD and in 29% LL rats. Granulesa-cell ovarian tumors developed in 24% LL rats, but not in the LD group. In another set of experiments female rats were administered intravenously with DMBA and in 4 weeks after the last injection of the carcinogen were exposed to constant illumination. In this condition the significant stimulation of mammary carcinogenesis has been observed: the number of mammary carcinomas per rat as well as their rate of growth was increased whereas the latent period was reduced as compared with the LD group.

A single intragastric administration of 50 mg DMBA at the age of 50 days induced mammary carcinomas in female Sprague-Dawley rats whereas the same dose of the carcinogen given to rats exposed to the LL from the age of 43 days was followed by the development secreting mammary fibroadenomas [33,34]. Ovaries of these rats were very susceptible to carcinogenic effect of DMBA and the increased conversion of androstendione into estradiol-17β was observed as well [35,36].

The incidence of mammary carcinomas in rats exposed to DMBA (20 mg orally) at the age of 55 days was 95% when animals were kept at the LL regimen and 60% at the LD regimen, $p < 0.001$ [37]. The number of the tumors per tumor-bearing rats was 2,26 and 1,13, and a tumor latency – 60±3.2 days and 94±8.3 days, in the LL and the LD groups, respectively, $p < 0.001$. Administration of melatonin inhibits development of DMBA-induced tumors in both groups.

Virgin female Sprague-Dawley rats were kept at the LL or at the DD regimens and were given with a single dose of DMBA (20 mg/rat) [38]. In the LL group the vaginal smears revealed a persistent estrus syndrome. In 6 months, progressively growing mammary carcinomas developed in 82% of rats in the LL group and in 87% of rats in the DD group. Additionally, 42% of the LL rats developed fibroadenomas of mammary gland whereas only 3% of the DD rats developed these tumors.

In our experiments [7] outbred Wistar-derived female rats were kept at the LD or the LL regimen from the age of 1 month. Since 2 weeks after this (at the age 45 days) rats of both groups were exposed to 3 weekly intravenous injection of NMU at a single dose of 50 mg/kg. The experiment was finished in 15 months after a start. Administration of NMU to the LD rats was followed by development of mammary adenocarcinomas in 55% of rats. The constant light exposure significantly promoted mammary carcinogenesis increasing the incidence and decreasing (2-fold) the latent period of development of mammary carcinomas (86±11.7 days in the LD rats versus 64±13.5 days in the LL rats, $p<0.01$). Rats exposed to the LL had much elevated night serum level of prolactin and decreased level of melatonin as compared with LD rats. Our data are in agreement with observations on promoting effect of the constant light on mammary carcinogenesis induced by DMBA (for review see [8,39].

Effect of light deprivation on mammary carcinogenesis

In a series of experiments Kuralasov [40,41] studied effect of light deprivation on growth and development of transplantable and DMBA-induced mammary tumors. Animals were kept in dark room (0 to 0.5 lux/cm^2) (DD) or under standard light-dark regimen (LD). The trasplantability of rat RMK-1 mammary carcinoma was 89.5% in the LD and only 58.6% in DD rats ($p < 0,05$). The mean doubling time of the tumor size was 82.3 hours in the LD group and 138 hours in the DD group. Administration of DMBA (2 mg x 4, i.v. weekly) induced mammary adenocarcinomas in 49% of the LD rats and in 5% of the DD rats. Number of tumors per tumor-bearing rat was 3.15 and 1.01 and latent period was 91±6.5 day and 135±10.5 days in the LD and in the DD group, respectively. Survival of tumor-bearing rats in the DD group was 54.7% longer than that in the LD group. The exposure to the DD regimen was followed by the decrease in serum levels of FSH, estradiol-17β and 11-oxicorticosteroides and by the decrease in ^{131}I uptake by thyroid gland in comparison to the LD rats [41].

Therapy with estrogen synestrol (0.2 mg/day) inhibited the growth of transplantable RMK-1 mammary carcinoma by 30.7% in the LD rats whereas by 88% in the DD rats [40]. In last group 36% of all tumors totally regressed, however no cases of total regression of the tumor was observed in the LD group. The exposure to the DD regimen potentiated the anti-tumor effect of some cytostatics and anti-cancer drugs (tamoxifen, androgens, tio-TEPA, cyclophosphamide, CMF, tio-TEPA+synestrol) [41].

This approach has been used for treatment of breast cancer patients (T_{2-4} N_{2-3} M_0) [41]. There are 138 patients under observation. Some of them (54 cases) were treated with polychemotherapy (modified Cooper's protocol+hormonotherapy). Forty six patients were exposed to radiotheray and 38 – to radiotherapy+chemotherapy. Premenopausal breast cancer patients revealed the decrease in the serum level of estradiol-17β (by 7-fold) and in the level of FSH, whereas the level of testosterone and progesterone increased as compared with the indices of the hormones in the same patients before the exposure to the constant dark regimen. In postmenopausal women these changes were less expressed than that in premenopausal ones. The dexamethasone test was negative in 62.5% of breast cancer patients kept in standard light/dark regimen and only in 9.5% of the patients kept in the darkness. Full or partial (> 50%) regression of breast carcinoma has been observed in 32.4% of patients of the control group and 78.6% in the "dark" group. The index of operability was 36% and 88% in the "light/dark" and "dark" groups, respectively [41].

Pinealectomy abolished the effects of the light deprivation on growth and function of hormone-dependent

tissues of reproductive system [11,14]. Blask et al. [42] administered DMBA into Sprague-Dawley rats and 3 weeks later rats were surgically blinded. The progressive tumor growth revealed 68% blinded and 88% of control rats ($p < 0.05$). Pinealectomy abolished the inhibitory effect of blinding on mammary carcinoma growth. Forty six percent of rats bearing DMBA-induced mammary tumors subjected to the surgical blinding and to an excision of olfactory bulbs revealed regression of mammary tumors whereas in control group no such cases have been observed [43]. If rats were subjected to the same surgery during the stage of initiation of the carcinogenesis, the inhibitory effect was much more expressed. Pinealectomy abolished the positive effect of blinding and anosmia [43]. Similar result were obtained by Sanchez-Barcelo et al. [44] in rats exposed to blinding plus anosmya before administration of DMBA or to this surgery after tumor development (1 cm in diameter).

In our experiments [7] female rats were kept at the LD, LL or DD regimens from the age 1 month. Two weeks later rats were exposed to 3 weekly injection of NMU at a single dose 50 mg/kg. The exposure to the DD regimen practically totally inhibited mammary carcinogenesis.

Thus, experiments with rodents give evidences of inhibitory effect of light deprivation on mammary carcinogenesis. There are some epidemiological data supporting these findings. Hahn [45] analyzed over 100,000 hospital discharge records published by the National Hospital Discharge Survey. Risk of breast carcinoma was twice less in primary blinded women as compared with sighted ones. In Sweden cohort study Feychtyng et al. [46] found risk of cancer to be lower among blind persons, and this reduced risk was also held true specifically for breast cancer in women. Pukkala et al. [47] and then Verkasalo et al. [48] observed the decrease of breast cancer risk in women with visual impairment (categorized from moderate low vision to total blindness) in Finland.

Effect of melatonin on mammary tumor development

The evidence of oncostatic action of melatonin on mammary tumor growth was obtained both in *in vitro* and *in vivo* experiments. MCF-7 human breast cancer cell line mostly used as a model for study of melatonin effect in vitro [49–51]. This cell lines originated from the pleural effusion of woman with metastatic breast carcinoma, and contains both estrogen and progesterone receptors. It was shown that a growth of MCF-7 cells is estrogen dependent and estrogens regulate the levels of some RNAs and proteins in them [50]. The inhibitory effect of melatonin on MCF-7 cells was firstly observed by Blask and Hill [52]. A lot of experiments on effect of melatonin on MCF-7 cell growth was reviewed recently by Cos and Sanchez-Barcelo [50,51]. It was shown that melatonin, at physiological concentration in a culture medium, inhibits cell proliferation and invasiveness of the cells, suppress mitotic activity of estradiol-17β and EGF, thus blocks proliferation at stage G_0/G_1 of mitotic cycle. Only those lines of MCF-7 and other breasts carcinoma cell lines mammary which have estrogen receptors (T47D, ZR75-1) have been shown susceptible to inhibitory effect of melatonin [49,50,53,54]. Lemus-Wilson et al. [55] observed inhibitory effect of melatonin on proliferative effect of prolactin and human growth hormone on MCF-7 cells. There are suggestion that melatonin could suppress autocrine and paracrine secretion of some growth factors (EGF, TGF-α, insulin, IGF-I and II [56].

Anisimov et al. [57] firstly have shown inhibitory effect of melatonin on transplantable mammary carcinoma RSM in female C3HA mice. Melatonin was given subcutaneously in a daily dose of 50 μg/mouse and 51% decrease of tumor size have been observed.

According Hamilton and Sneddon [33], morning administration of melatonin to rats was followed by the increase in the incidence of mammary carcinomas induced by 7,12-dimethylbenz[a]anthracene (DMBA) in female rats. However in a plenty of subsequent experiments it was shown that the evening treatment with melatonin inhibits DMBA-induced mammary carcinogenesis [58–64]. Melatonin also attenuated the stimulating mammary carcinogenesis effect of pinealectomy [65]. Another mammary carcinogen, N-nitrosomethylurea (NMU) was also used in experiments with melatonin. This model has some advantages in comparison to DMBA-induced carcinogenesis. In contrast to DMBA-induced mammary adenocarcinoma susceptible mostly to prolactin, the growth of NMU-induced mammary carcinoma are more depend on the levels of estrogens, that is more relevant to human breast carcinoma [66]. In several studies have been observed inhibitory effect of melatonin on NMU-induced carcinogenesis in mammary gland of rats [60,67,68]. It is interesting that melatonin failed reveal the dose-dependent inhibitory effect in this model. The effect of melatonin was similar to those of estrogen antagonist tamoxifen [67].

The data on effect of melatonin on chemically-induced mammary carcinogenesis are summarized in the Table 1.

Subramanian and Kothari [71] reported a suppressive effect of melatonin on the development of spontaneous mammary carcinomas in female C3H/Jax mice treated from the age of 3 weeks until the age of 12 months. In female CBA mice, administration of melatonin with night drinking water from the age of 6 months failed change the incidence of spontaneous mammary adenocarcinomas [72].

The oncostatic effects of melatonin on the mammary gland have been studied in transgenic mice carrying the N-*ras* proto-oncogene under the control of the MMTV-LTR [73]. Female (4-week-old) virgin mice with positive transgenic pedigrees were injected with melatonin (200 μg/mouse/ day, 5 times a week) or vehicle late in the evening. After 5 months of treatment, animals were sacrificed and the mammary glands were dissected for whole mounts, histology, and immunohistochemical analysis with a mouse monoclonal antibody specific for N-*ras* protein. Mammary glands of control transgenic mice showed different densities of hyperplastic alveolar nodules (HANs) consisting primarily of dysplastic epithelial cells with nuclear atypia and prominent nucleoli. The epithelial cells of HANs showed a high expression

Table 1. Effect of melatonin on development of mammary carcinoma induced by chemical carcinogens in female rats

Strain	Carcinogen treatment	Photo-period	Melatonin	Effect	References
Sprague-Dawley	DMBA, 30 mg/rat, per os, at the age of 50 days	12:12 from the age of 43 d.	100 μg/day from the age of 43 days	↑ tumor incidence	[34]
Sprague-Dawley	DMBA, 25 mg/rat, per os, at the age of 60 days	12:12	200 μg/day from the age of 60 days	↓ tumor incidence	[58]
Sprague-Dawley	DMBA, 15 mg/rat, per os, at the age of 50 days	12:12	500 μg/day from the age of 50 days	↓ tumor incidence	[65]
Holtzman	DMBA, 10 mg/100 g of b.w., per os, at the age of 55–60 days	10:14	500 μg/day, 52–145 days	↓ tumor incidence	[59]
Sprague-Dawley	DMBA, 5 mg/rat, i.v. at the age of 55 days	12:12	250 μg/day from the age of 55 days	↓ tumor incidence	[63]
Holtzman	DMBA, 20 mg/rat, per os, at the age of 55 days	10:14	100 μg/day from the age of 55 days	↓ tumor incidence	[9]
Sprague-Dawley	NMU, 50 mg/kg x 2, i.v., at the age of 50 and 57 days	12:12	250 μg/day from the age of 55 days	↓ tumor incidence	[67]
Sprague-Dawley	DMBA, 10 mg/rat, per os, at the age of 55 days	10:14	200 μg/day from the age of 55 days	↓ tumor incidence	[64]
Holtzman	DMBA, 10 mg/rat, per os, at the age of 55 days	10:14	200 μg/day from the age of 55 days	↓ tumor incidence	[69]
Fischer	Grafting of DMBA-induced mammari carcinomas	12:12	100 μg/day from the age of 56 days	↓ tumor size	[70]
Sprague-Dawley	NMU, 50 mg/kg of b.w., i.v., at the age of 50 days	12:12	200 μg/day from the age of 50 days	↓ DNA synthesis in mammary epithelium	[61]
LIO	NMU, 50 mg/kg of b.w., i.v., at the age of 60 days	14:10	20 mg/l of drinking NMU water at night time 2 days before and 1 day after	↓ tumor incidence	[68]

of N-*ras* while no immunostaining was detected in the unaffected mammary parenchyma. Only one (10%) of the control transgenic mice presented an infiltrating ductal carcinoma with the neoplastic cells overexpressing N-*ras* protein. The mammary glands of melatonin treated mice had a lower density of HANs, absence of epithelial dysplastic cells, and weak immunostaining of N-*ras* protein in comparison to the vehicle-treated group. None of the melatonin treated animals developed mammary carcinomas during the observation period. The lymph nodes of the inguinal mammary glands of all the vehicle-treated transgenic mice presented hyperplasia and two animals even had lymphomas, whereas in melatonin-treated animals there was less hyperplasia (two cases were atrophic) and a lack of lymphomas. Authors conclude that in the mammary glands of MMTV-LTR/N-ras transgenic female virgin mice, melatonin reduces the incidence of HANs and the expression of N-*ras* protein in focal hyperplastic lesions, completely prevents the development of epithelial cell atypia and mammary adenocarcinomas, and also reduces the hyperplasia of the mammary lymphoid tissue and prevents the development of lymphomas.

Four-week-old TG.NK female mice with MMTV/c-neu oncogene were gavaged with melatonin at 50, 100 or 200 mg/kg dissolved in corn oil [74]. Melatonin delayed the appearance of palpable mammary tumors and the growth of tumors with a dose-related statistically significant negative trend for the incidence of tumors.

The administration of melatonin during the night time significantly decreased the incidence of mammary adenocarcinomas in FVB/N female mice transgenic for oncogene HER-2/neu in the LD group and, in a lower manner, in the LL group, $p<0.0003$ [31]. The mean number of tumors per tumor-bearing mouse was not changed by the melatonin treatment in both light regimens. However, at the LL regimen the number of mice bearing 4 and more tumors was reduced by melatonin more significantly than that in mice kept at LD regimen ($p<0.05$). Melatonin administration decreased the size of mammary adenocarcinomas ($p<0.05$) and the incidence of lung metastases ($p<0.07$) at the LD regimen, but not at the LL regimen as compared with the LD and LL groups not exposed to melatonin, respectively. In order to evaluate whether the decreased incidence of mammary tumors observed in mice kept in standard light/dark regimen was due to an effect of melatonin on mammary gland, we performed RT-PCR analysis for HER-2/neu gene expression in the mammary tumors from mice of the LD group. mRNA for HER-2/neu gene was greatly expressed in saline treated mice whereas it was significantly (2.5-fold) decreased in animals chronically treated with melatonin [31].

The data on effect of melatonin on mammary carcinogenesis in inbred and transgenic mice are summarized in the Table 2.

Fourty patients with failed metastatic breast cancer were randomly assigned to receive tamoxifen alone or tamoxifen plus 20 mg of melatonin per os, at 8 p.m. At 1 year, 12 from 19 melatonin-treated patients were alive (63.2%) compared to 5 from 21 patients treated with tamoxifen alone (23.8%, $p < 0.01$) [75–77].

Table 2. Effect of melatonin on mammary carcinoma development in inbred and transgenic mice

Strain	Photo-period	Melatonin	Effect	References
C3H/Jax	12:12	25 μg/mouse from the 45th day of life	↓ tumor incidence	[71]
Transgenic ras-MMTV-LTR	14:10	200 μg/day from the age of 28 days	↓ expression of *ras* protein, density of hyperplastic nodules in mammary gland	[73]
Transgenic TG.NK (MMTV/c-neu)	12:12	50–200 mg/kg per os with corn oil from the age of 28 days	↓ tumor incidence and weight	[74]
Transgenic HER-2/neu	12:12	20 mg/l of drinking water at night time from the age of 60 days	↓ tumor multiplicity	[31]

Mechanisms of anti-carcinogenic effects of melatonin

In rats exposed to a single intragastric dose of DMBA at the age of 51 days nocturnal plasma melatonin was found to be significantly depressed both 2 and 7 days after the carcinogen administration (–37% and –31%, $p < 0.05$), whereas the main periferal metabolite of melatonin, 6-sulfatoxymelatonin (aMT6s), did not differ compared to controls, indicating an increased degradation of the pineal hormone due to DMBA [78,79]. Later it was shown that the hepatic micirosomal monoxygenases of the cytochrome P450 system catalyzing the 6-hydroxylation of melatonin are also enhanced by DMBA treatment [80]. Comprehensive review on the melatonin level and rhythm in breast cancer patients has been recently published by Bartsch et al. [81]. In brief, nocturnal circulating melatonin is diminished in patients with breast cancer. The most prominent depletion are found among patients with advanced localized primary tumors leading to a negative correlation between circulating melatonin level and tumor size. As it was reviewed earlier, exogenous melatonin can prevent development of mammary cancer. The mechanisms of the inhibitory effect of melatonin on mammary carcinogenesis include the variety possibilities, discussed in several comprehensive reviews [39,81,82]. In the Table 3 most important mechanisms are summarized.

Table 3. Assumptive mechanisms involved in inhibitory effect of melatonin on mammary tumor development

Parameters	Effect	References
Gonadotropins (FSH and LH) level	↓	[83]
Prolactin level	↓	[7,59]
Growth hormone, IGF-I	↓	[74]
Insulin level	↓	[84]
Estrogen level	↓	[85]
Estrogen receptor expression	↓	[85]
Calcium/calmodulin activity regulation	↓	[85]
Mutagenic effect (DMBA, irradiation)	↓	[86,87]
Clastogenic effect (NMU)	↓	[68,86]
DNA adduct formation	↓	[88]
Proliferative activity	↓	[39,50,51]
Protein kinase C	↓	[89,90]
Micritubule polymerization	↓	[90]
Apoptosis	↑	[90]
ROS formation	↓	[88]
Antioxidative defence system	↑	[88]
Linoleic acid uptake	↓	[39,74,89,90]
13-hydroxyoctadecadienoic acid formation	↓	[89,90]
Immune surveillance	↑	[92]
Gene expression of immunomodulating cytokines	↑	[93]
HER-2/neu expression	↓	[31]
ras expression	↓	[73]

It is clear that the majority of the listed factors in the more or less degree might be involved in the inhibitory effect of melatonin on the development of mammary carcinoma.

Conclusion

Data reviewed show the important role of the pineal gland in development of breast cancer. Inhibition of pineal function with the pinealectomy or with exposure to the constant light regimen stimulates mammary carcinogenesis, whereas light deprivation inhibits the carcinogenesis. Epidemiolgical observations on increased risk of breast cancer in night shift workers, flight attendants, radio and telegraph operators and on the decreased risk in blind women are in accordance with the results of experiments in rodents. Treatment with pineal indole hormone melatonin inhibits mammary carcinogenesis in pinealectomized rats or animals kept at the LD or the LL regimens. It is important to note that light/dark regimen influence carcinogenesis in some other organs. In our experiments it was shown that in the exposure to constant light significantly promoted the transplacental carcinogenesis induced by N-nitrosoethylurea in rats whereas the exposure to constant darkness inhibited it [94]. Female BDII/Han rats reveal extremely high (up to 90%) incidence of the spontaneous development of endometrial adenocarcinomas. The exposure to the LL regimen started at the age of 30 days was followed by significant shortening of animals' life span, due to acceleration of tumorigenesis [95]. However, the exposure to the LL started at the age of 50 days was not effective. Administration of melatonin to BDII/Han rats inhibited the development of endometrial adenocarcinomas, but was less effective being started at the age 50 days and was absolutely non-effective when was started at the age of 6 months. There is evidence of promoting effect of LL regimen on diethylnitrosamine-induced hepatocarcinogenesis in rats [96] and growth of transplantable rat hepatoma 7288CTC [89,90]. On the other hand, melatonin inhibits growth of transplantable murine colon tumors

(Colon 38 and CT-26) [97,98], inhibits colon carcinogenesis induced by 1,2-dimethylhydrazine in rats [99,100] and cervico-vaginal carcinogenesis induced by DMBA in mice [101].

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