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Review

REVIEW

Melatonin as a promising agent to treat ovarian cancer: molecular mechanisms

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Abstract

Ovarian cancer (OC) has the highest mortality rate of all gynecological cancers, and most patients develop chemoresistance after first-line treatments. Despite recent advances, the 5-year relative survival is ~45% for all OC subtypes, and invasive epithelial OC has only a 17% survival rate when diagnosed at a late stage. Identification of new efficacious molecules or biomarkers represents important opportunities in the treatment of OC. The pharmacological and physiological properties of melatonin indicate this agent could be useful against OC progression and metastasis. In normal cells, melatonin has potent antioxidant and anti-apoptotic actions. Conversely, melatonin has pro-oxidant as well as anti-proliferative, anti-angiogenic and immunomodulatory properties in many cancer types including hormone-dependent cancers. Although melatonin receptors have been identified in OC cells, the exact mechanism by which melatonin induces anticancer activities remains incompletely understood. Clinical studies have reported negative correlation between aggressiveness of OC and serum levels of melatonin, reinforcing the idea that melatonin may be a critical factor determining OC development. *In vitro* and *in vivo* studies suggest melatonin differentially regulates multiple signaling pathways in OC cells. This focused review explores the potential mechanisms of action of melatonin on cultured OC cells and in experimental models of OC in an attempt to clarify how melatonin modulates the signaling pathways involved in cancer cell apoptosis, survival, inflammation, proliferation and metabolic processes. Based on the evidence presented, we feel that melatonin, as an agent that controls cellular signals associated with malignancy, may be beneficial in combination with other therapeutics for OC treatment.

Introduction

A brief description of molecular aspects involving the anticancer properties of melatonin

A variety of new agents has been proposed to counteract ovarian cancer (OC) as adjuvant therapeutic strategies, including melatonin. Melatonin (N-acetyl-5-methoxytryptamine) is a small lipophilic indoleamine produced by the pineal gland and by extrapineal tissues (e.g. ovary, retina, gastrointestinal tract). In healthy cells, melatonin prevents apoptosis through a variety of well-recognized mechanisms (1). On the contrary, it has been consistently reported to exert antiproliferative, anti-angiogenic, pro-apoptotic and immunomodulatory properties in many cancer types including OC (2).

Molecular mechanisms by which melatonin exhibits its oncostatic action include the regulation of estrogen receptor expression, protein kinases activities, calcium/calmodulin signals, cellular redox state, cytoskeletal reorganization and function, melatonin receptor-mediated cell signaling, fatty acid metabolism and suppression/activation of the intracellular signal transduction (3). In addition, melatonin exerts anticancer effects through its anti-metastatic potential, drug sensitivity restoration, apoptosis induction, growth inhibition and anti-angiogenic and anti-invasive actions (4). Importantly, Reiter *et al.* (5) extensively summarized the literature showing that melatonin mitigates a number of cancer types at the initiation, progression and metastasis levels; the processes by which

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Abbreviations

5-FU	5-fluorouracil
AKT	protein kinase B
ATO	arsenic trioxide
Bax	apoptosis regulator
Bcl-2	family of apoptosis regulators
Bid	BH3 interacting-domain death agonist
B-Raf	proto-oncogene
CaM	calmodulin
CaMKII	Ca2+/calmodulin-dependent protein kinase II
cAMP	cyclic adenosine monophosphate
CDDP	*cis*-Diamminedichloroplatinum
CDKs	cyclin-dependent kinases
CLOCK	clock circadian regulator
c-Myc	Myc proto-oncogene protein
DMBA	7,12-Dimethylbenz[a]anthracene
ECM	extracellular matrix
EMT	epithelial-mesenchymal transition
ERK1/2	extracellular signal-regulated protein kinases 1 and 2
FOXO1	forkhead box protein O1
GRP78	78-kDa glucose-regulated protein
GSK3β	glycogen synthase kinase 3β
GTPase Kras	proto-oncogene
HDAC4	histone deacetylase 4
HER	epidermal growth factor receptor
HIF-1α	hypoxia-inducible factor 1-alpha
HSP27	heat-shock protein 27
hTERT	human telomerase reverse transcriptase
IFN-β	interferon β
IGFBP-3	insulin-like growth factor binding protein-3
IkBα	inhibitor of NFkB alpha
IKK-α	IkB kinase alpha
IL-6	interleukin 6
iNOS	inducible nitric oxide synthase
IRF3	interferon regulatory factor 3
LDH	lactate dehydrogenase
MAPK	mitogen-activated protein kinase
miRNA	microRNA
MKP3	MAP kinase phosphatase 3
MLCK	myosin light-chain kinase
MMP	metalloproteinase
MT1	melatonin receptor 1
MT2	melatonin receptor 2
mTOR	mammalian target of rapamycin
MyD88	myeloid differentiation factor 88
NF-κB	nuclear transcription factor-kappa B
OC	ovarian cancer
OSE	ovarian surface epithelium
P21	cyclin-dependent kinase inhibitor 1
p53	tumor suppressor protein
p65	phospho-NF-κB subunit p65
p90RSKs	phosphorylating 90-kDa ribosomal S6 kinases
PARP	Poly (ADP-ribose) polymerase
PDI	protein disulfide isomerase
PDT	photodynamic therapy
PER2	period circadian clock 2
PI3K	phosphoinositide 3-kinase
p-JNK	phospho-c-Jun N-terminal Kinase
p-p38	phospho-p38 MAPK
PPAR-γ	peroxisome proliferator-activated receptor gamma
pRb	retinoblastoma protein
PTEN	phosphatase and tensin homolog
ROCK	Rho-associated protein kinase
ROS	reactive oxygen species
RSKs	ribosomal S6 kinases
RZR/ROR	orphan receptors family
SENP1	sentrin-specific protease 1
Sirt1	sirtuin 1
TGFβ-1	transforming growth factor beta-1
TLRs	toll-like receptors
TNF-α	tumor necrosis factor alpha
TP53	p53 gene
TRAF6	TNF receptor-associated factor 6
TRIF	TLR-associated activator of interferon
VEGF	vascular endothelial growth factor
VEGFR2	vascular endothelial growth factor receptor 2

melatonin restrains cancer development and growth have been often described, whereas other roles appear to be merely epiphenomena of a more complex action of melatonin that should be thoroughly investigated.

Metastasis is a hallmark of cancer aggressiveness and a major cause of death. It is well-known that to reach the metastatic phenotype the tumor cells use a number of molecular mechanisms including modulating cell adhesion, cytoskeletal organization, extracellular matrix (ECM) interactions, epithelial–mesenchymal transition (EMT) and angiogenesis (4). A key characteristic of a cell in the metastatic process is overcoming cell–cell and cell–ECM adhesions. E-cadherin and occludin are important components of adherens and tight junctions, respectively, and their loss favors tumor metastasis (6,7). Studies using breast (8), gastric (9) and lung (10) cancer cells have shown that melatonin upregulates these proteins, thus reverting their invasive status. Like other cell adhesion proteins, integrins are heterodimeric molecules involved in cell–ECM communication and also influence the migratory potential of cancer cells. In recent studies, melatonin was reported to act against metastasis by reducing the expression of αvβ3 integrin in glioma cells (11) and upregulating β1 integrin in breast cancer cells (12).

The cross talk between cadherins and integrins is mediated by the Rho-associated protein kinase (ROCK) and myosin light-chain kinase (MLCK), two key kinases responsible for cytoskeletal reorganization (13). Melatonin is effective by increasing anchorage of invasive cancer cells by organization of ROCK-regulated microtubules and microfilaments (14), and downregulating MLCK and ROCK (10,15). Cell migration to a secondary site also depends on ECM remodeling mediated by matrix metalloproteinases (MMP). In a gastric adenocarcinoma cell line, melatonin inhibited MMP-9 activity by binding and interacting with its active site (16). Additionally, melatonin targeted cytoplasmic Akt/ERK/JNK pathways by reducing MMP-9 transcription dependent of nuclear factor kappa B (NF-κB) (17).

The transformation process of a cell from its adherent epithelial state to a migratory mesenchymal phenotype involves rearrangement of cytoskeleton, loss of apical-basal polarity and cell–cell junctions and is mediated by a number of signaling pathways, such as NF-κB, Wnt, Notch, Hedgehog and AP-1 (4,18). NF-κB transactivation can influence the EMT by interacting with transcription factors (Snail, Slug, Twist and Zeb) which downregulate E-cadherin (19) and by induction of vimentin expression, which favors mesenchymal cell transformation (20,21). Melatonin is a promising agent that interferes with NF-κB signaling, even decreasing vimentin expression in breast cancer cells (22). Melatonin also modulates the activation of glycogen

synthase kinase 3β (GSK3β), a component of the destruction complex, formed in the absence of Wnt pathway signaling, thus inducing β-catenin dissociation and, consequently, stopping the EMT (23).

Drug resistance is one of the biggest challenges regarding cancer, particularly in ovarian carcinomas; this enhances patient's morbidity and mortality and reduces the efficacy of standard chemotherapies. When used as an adjuvant therapeutic, melatonin enhanced HeLa cervical cancer cells sensitivity to cisplatin through accumulation of damaged DNA and, consequently, increasing the apoptosis ratio (24). Studying colorectal cancer cells, Gao *et al.* (25) observed that melatonin enhanced the chemotherapeutic effects of 5-fluorouracil (5-FU), one of the most common agents used to treat colorectal cancer; this involved inhibition of NF-κB/iNOS, E-cadherin/MMP9, PI3K/AKT and caspase/PARP pathways. Notably in these cells, melatonin demonstrated a wide range of actions such as anti-proliferative, pro-apoptotic and anti-migration.

Melatonin inhibits tumor growth and induces apoptosis in various types of cancer. In prostate cancer models (LNCaP cell line and TRAMP murine model), melatonin induced antitumor activities dependent on IGFBP3 and ERK1/2 signaling; in these cells, melatonin blocked the translocation of androgen receptor, thus documenting its anti-androgenic effects (26). An earlier study by Quintana *et al.* (27) demonstrated that melatonin, at a 1 mmol/l concentration, was sufficient to improve the cytotoxicity of hyperthermia in U937 human leukemia cells; the indole enhanced the apoptotic effect through activation of caspase-2, -3, -8 and -9, in addition to increasing hyperthermia-induced Bid activation, Bax translocation and cytochrome c release. Following the treatment of rhabdomyosarcoma cell lines with the indoleamine, Codenotti *et al.* (28) observed fragmentation of DNA and disruption of cell membranes, changes commonly found in apoptotic cells. Using SGC7901 gastric cancer cells, a recent study by Li *et al.* (29), described the involvement of melatonin in increasing the expression of phosphorylated (p)-p38 and p-JNK proteins, in addition to a decrease in the level of p65. To achieve significant efficacy on the apoptotic index following 24 h of treatment, the optimal dose of melatonin was determined to be 2 mM. In pancreatic cancer, melatonin induces apoptosis through a series of mechanisms, which include mitogen-activated protein kinase (MAPK) pathways; this results in an increased activation of JNK and ERK, suppression of NF-κB, overexpression of caspase-3 and phosphorylation of apoptosis-related proteins like Bax and Bcl-2 (30). By enhancing the expression of p21 and the Bax/Bcl-2 ratio, melatonin triggers apoptosis in breast cancer cells (31). Melatonin also induces apoptosis in LoVo colorectal cancer cells by binding to calmodulin (CaM), and then acting as an antagonist of the Ca2+/calmodulin-dependent protein kinase II (CaMKII) activity, which in turn induces the translocation of the histone deacetylase 4 (HDAC4) to the nucleus (32).

When administered alone or in combination with other agents, melatonin has already been shown to be effective against cancer, acting in synergism with standard and novel anticancer therapies to counteract tumor growth and angiogenesis. Indeed, when melatonin was used as an adjuvant for arsenic trioxide (ATO) treatment, it inhibits the growth of breast cancer cells via induction of p21 expression, leading to G_1 cell cycle arrest and suppression of human telomerase reverse transcriptase (hTERT) and Myc proto-oncogene protein (c-Myc) (31). Using melatonin to treat gastric cancer cells with overexpressed nuclear RZR/ROR receptors, Wang *et al.* (33) reported its anti-angiogenic and growth inhibitory effects. In this case, the indoleamine reduced the expression of RZR/ROR receptors and targeted vascular endothelial growth factor (VEGF), hypoxia-inducible factor 1-alpha (HIF-1α) and sentrin-specific protease 1 (SENP1), which are essential to angiogenesis and the spread of tumorigenic cells. In pharmacological concentrations, melatonin is effective in inhibiting HIF-1α and VEGF expression in HepG2 hepatocarcinoma cells, thus reducing its invasive potential (34). Recently, González *et al.* (35) showed that melatonin inhibits VEGF expression and secretion in SH-SY5Y human neuroblastoma cells co-cultured with endothelial cells. Importantly, the destabilization of the transcriptional factor HIF-1α and, consequently, the reduction of VEGF expression seem to be the major events by which melatonin acts against angiogenesis; however, its effects involving the reduction of the vasoconstrictor endothelin-1 via forkhead box protein O1 (FoxO1) and NF-κB inactivation and inhibition of the ERK/Akt/NF-κB pathway have also been proposed (4).

A series of novel studies clarified the role of melatonin in influencing gene expression, particularly acting as an epigenetic modulator. Hardeland (36) summarized different possible epigenetic mechanisms of melatonin regulating both core and accessory oscillating genes, such as *Per2* or *Clock* and *Sirt1* and *PPAR-γ*. Additionally, a new perspective of melatonin effects against cancer has been suggested by reports of miRNA regulations on breast cancer cells (37,38). More specifically, melatonin treatment effectively downregulated miR-24, an important oncogenic miRNA that reduces the activity of p38–p53 axis components involved in DNA repair and inhibition of cell proliferation (38). Curiously, these results may indicate the signaling pathway by which melatonin enhance p53 expression after activation of its receptors, MT1 and MT2, a condition that was previously reported by Santoro *et al.* (39).

Given that multiple molecular pathways have been uncovered related to the anticancer effects of melatonin, a complete understanding on how this indoleamine properly orchestrates such functions is yet far from being fully established.

Ovarian cancer: origin, subtypes and conventional treatments

Among gynecological malignancies, OC remains the leading cause of death worldwide (40). Unfortunately, OC presents with a poor prognostic outcome for patients diagnosed at a late stage (with a 5-year survival rate of 35%), and there is no screening method for early detection. The primary OCs are classified into epithelial, mesenchymal, germ cell and sex cord-stromal origin. About 90% of OC subtypes originate from ovarian surface epithelium (OSE) or even from distal fallopian tube epithelial cells, with ~70% of cases diagnosed with widespread metastasis (41). These malignant OCs are further subclassified into serous, mucinous, endometrioid and clear cell carcinomas (42), and show particular features of its pathophysiological, genetic and molecular components (43). To date, debulking surgery with chemotherapy or neoadjuvant chemotherapy followed by interval debulking represent the gold standard treatment for OC. New promising therapeutic results have been achieved with the use of cytotoxic agents (44). Notably, late diagnosis and the chemoresistance to conventional treatments (platinum- and taxane-based therapies) represent a big limiting factor for treatment, as many women relapse with aggressive disease. Although additional therapies using chemical agents (aromatase inhibitors, anti-estrogens, anti-angiogenic drugs etc.) are recommended for such OC subtype, they are still very far from optimal efficacy (41).

The malignant epithelial OCs that are amenable and confined to the ovary for a considerable period of time belong to

the Type I group (<25%). Type II tumors are mostly invasive and often spread early to extraovarian regions during their development (45). Although increasing attention has been given to the new interpretation of the etiology of OC based on the integration of multivariate biomarkers, ultrasound and imaging/screening diagnostics (46), the lack of shared genetic mutations involving specific signaling pathways makes it difficult to extrapolate proper target-based therapies for OC. Although molecular alterations involving specific genetic mutations (e.g. TP53, BRCA1 and 2, proto-oncogene B-Raf and GTPase Kras) are critical for the development of different OC subtypes, epidemiological factors, including exposure to steroid hormones, number of pregnancies, age at menarche and menopause, and hormone replacement therapy, are also relevant to OC risk and management (47,48).

It is important to note that drugs targeting specific molecular signatures in tumor cells could be used alone or in combination with existing therapies to ensure clinical improvement for women with OC. Therefore, therapeutic modalities using OC cells or animal models of OC that recapitulate their human counterparts are urgently needed.

Based on current evidences, melatonin' actions can be reproduced with significant implications in both prevention and treatment of tumors, including OC. The variability of its *in vitro* anticancer effect depends on the conditioning of the cell culture medium, cell differentiation and sensitivity to oncogenic particles; melatonin' *in vivo* effects are surely complex and multifaceted into the wide biological context (49). Based in the properties of melatonin, the molecular dynamics on how a normal or tumor cell divides are also dependent on successive events that involve the action of melatonin on circadian time-markers (3).

General overview of the role of melatonin on OC

In normal ovary, melatonin is secreted by the granulosa cells of preovulatory follicles. In addition, melatonin treatment influences sex steroid production essential for ovulation, luteal function and oocyte quality (50). Melatonin is a powerful free radical scavenger and stimulates the activity of antioxidant enzymes in the ovary (51–53). To explain the primary origin of OC, a recent study revealed that generation of reactive oxygen species (ROS) during ovulation might be responsible for the transformation of tubal fimbria cells with p53 loss, whereas melatonin abolished the tumorigenic effect induced by ROS (54). In support of these findings, a retrospective study involving 277 women with OC showed low serum levels of melatonin compared with those in healthy women (41.8 versus 82.4 pg/ml, respectively). Even though melatonin levels in perimenopausal and postmenopausal women exhibit only minor changes (55), there may be a protective effect of melatonin on the development of OC during this reproductive stage. Although no clear relationship between a woman's urinary melatonin level and OC risk exists (56), *in vitro* and *in vivo* experimental evidence indicates that melatonin may represent a significant therapy against OC. Figure 1 summarizes the most general processes that are regulated by melatonin in different OC subtypes.

Melatonin has well-described actions via MT1/MT2 membrane receptors, nuclear receptors (ROR/RZR), calmodulin binding and as a ubiquitous free-radical scavenger (57). Recent advances showed that MT1 activation is responsible for the oncostatic role of melatonin, which results in inhibition of cAMP synthesis and depletion of protein kinase A, C and MAPKs (58). This regulation can negatively influence the expression of genes involved in angiogenesis, proliferation and metastasis after preventing the phosphorylation of a transcription factor dependent on cAMP activity (57). Interestingly, the expression of MT1 is higher in normal ovarian IOSE 364 cells than in OC SKOV-3 and OVCAR-3 cells (58), and exogenous nanomolar concentrations of melatonin upregulate MT1 expression (59). These latter workers also described an intimate relationship between melatonin and estrogen receptors, where estrogens downregulate melatonin receptors and melatonin downregulates estrogen receptors. MT1 is generally found to be reduced in serous papillary OC, and recently, we identified an upregulation of MT1 levels in OC-bearing animals following melatonin treatment (41). These data suggest an important, albeit not fully understood, role of melatonin associated to MT1 pathway in oncogenesis, particularly in OC. Because of its low density, the function and pharmacology of melatonin receptors have been difficult to define in a variety of animal tissues, especially for the MT2 (60). Considering the efficacy and lack of selectivity of the ligands, melatonin or its analogues can act by simultaneous activation of MT1/MT2 heterodimers expressed in a variety of cells; this could promote additive, synergistic or even antagonist actions (61). This is important for cancer prevention and inhibition, as discussed by Liu *et al.* (60), who reported that MT1 and MT2 agonists inhibited the proliferation of breast cancer MCF-7 cells, whereas the antiproliferative effect of melatonin was abolished following treatment with MT1 and MT2 antagonists.

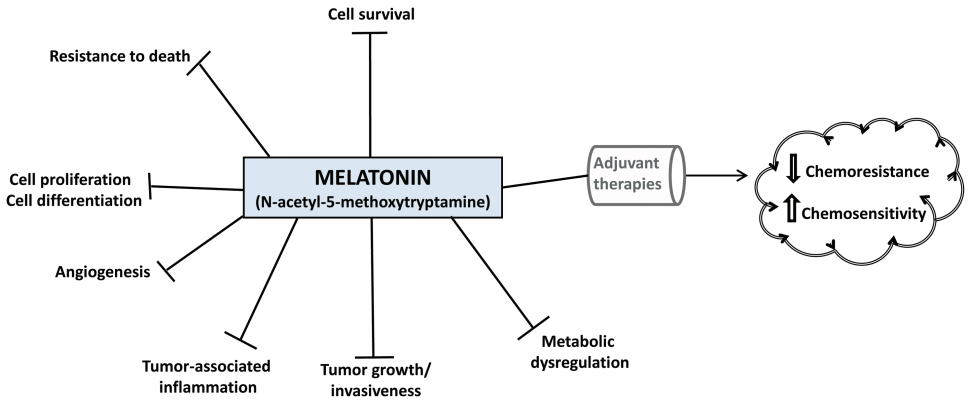

Figure 1. The figure summarizes some processes whereby melatonin has inhibitory action on important molecular mechanisms involved in OC progression. Additional effects of melatonin are also expected following its co-administration with other standard chemotherapeutics (e.g. platinum compounds and taxol derivatives) thereby reducing resistance and improving chemosensitivity to treatment.

Specific tumorigenic pathways targeted by melatonin in OC cell

Figure 2 illustrates the possible signaling pathways by which melatonin probably differentially regulates important cellular events in OC. Melatonin has been described as a potent oncostatic agent with pro-apoptotic, antioxidant and anti-angiogenic effects (62). The oncostatic effect of melatonin was previously reported by Petranka *et al.* (63) using an ovarian carcinoma cell line (BG-1); in this work, melatonin and the CGP52608, a melatonin nuclear orphan receptor (RZR) agonist, were effective in reducing cell growth in addition to having an impact on apoptosis.

Treatment with melatonin, varying from 400 to 600 μM, reduced the survival and proliferation rates of two OC cell lines (OVCAR-429 and PA-1), increasing the number of cells in G_1 phase and decreasing the number in S phase of the cycle (64). Previously, Kim *et al.* (65) reported an elevated number of SKOV-3 cells in G_1 phase after use of melatonin as a cotreatment with cisplatin. Cyclin-dependent kinases (CDKs) have key roles in cell cycle during phosphorylation of the retinoblastoma protein (pRb). Melatonin efficiently downregulated CDKs, especially CDK2 and 4, in two OC cell lines, thus triggering cell cycle arrest (Figure 2). Additionally, melatonin enhanced the levels of p53, a classical tumor suppressor protein, and p27, a member of CIP/KIP family that inhibits complexes to allow pRb phosphorylation (64). According to Futagami *et al.* (66), the antiproliferative effect of melatonin has also been shown by its ability to increase the sensitivity to *cis*-diamminedichloroplatinum (CDDP) in two human OC cell lines (HTOA and OVCAR-3). When associated to CDDP, melatonin at physiological concentrations of 10^{-6} and 10^{-9} exhibited an antiproliferative effect in HTOA and OVCAR-3 cells, respectively; in the OVCAR-3 cells, melatonin further reduced telomerase activity, thus suggesting that melatonin changes anti-cancer drug sensitivity and cell survival. Recently, the interplay between melatonin and different extracellular matrices has indicated different responses on proliferation and stemness of SKOV-3 cells. Through its anti-invasive activities, melatonin (0.1 mM) decreased the number of viable cells, colony formation and percentage of stem-like cells in all available matrices (fibronectin, gelatin, matrigel and collagen); these cellular behaviors are thought to have an involvement with upregulation of E-cadherin and downregulation of VEGF (67).

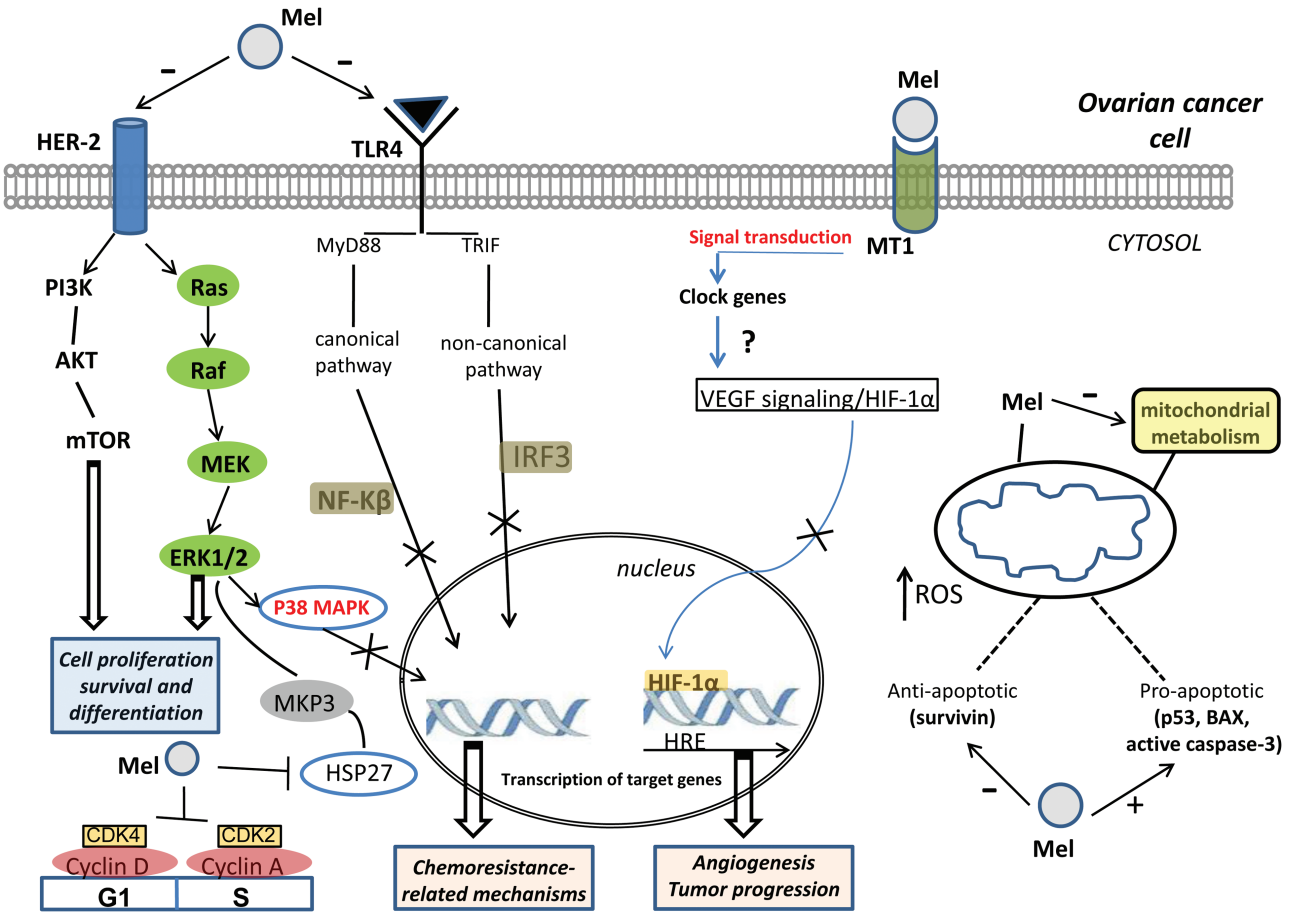

Figure 2. Schematic representation for the role of melatonin at different therapeutic targets in OC cells. Melatonin has both membrane and intracellular actions that lead to inhibition of cell proliferation, survival, migration, inflammation and angiogenesis of OC. This regulation may directly involve intracellular targets or it may occur indirectly via MT1 receptors. Negative or positive signals indicate downregulation or upregulation resulting from melatonin therapy, respectively. X identifies pathways that are blocked by melatonin. Mel, melatonin; MT1, melatonin receptor 1; ROS, reactive oxygen species; p53, tumor suppressor protein p53; BAX, Bcl-2-like protein 4; NF-kB, nuclear factor kappa B; IRF3, interferon regulatory factor 3; TLR4, toll-like receptor 4; MyD88, myeloid differentiation factor 88; TRIF, TIR-domain-containing adapter-inducing interferon-β; VEGF, vascular endothelial growth factor; HIF-1α, hypoxia-inducible factor 1α; HER-2 human epidermal growth factor receptor 2; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; Ras/Raf, protein kinases; MEK, p38MAPK, P38 mitogen-activated protein kinase; ERK1/2, extracellular-signal regulated kinases 1 and 2; MKP3, mitogen-activated protein kinase phosphatase-3; HSP27, heat-shock protein 27; CDK2, cyclin-dependent kinase 2; CDK4, cyclin-dependent kinase 4; G1 and S, phases of the cell cycle.

Extracellular signal-regulated kinase (ERK) is activated by Raf and is responsible for phosphorylating 90-kDa ribosomal S6 kinases (p90RSKs) for anti-apoptotic regulation (68). RSKs are expressed in a number of cancer types where they are closely related to tumorigenesis (69,70). In addition to their anti-apoptotic role, RSKs can directly interact with heat-shock protein 27 (HSP27) to promote cancer cell survival (65). When administered alone, melatonin (2 mM) does not interfere with p90RSKs and HSP27 levels, but did accelerate HSP27 dephosphorylation in SKOV-3 cells treated with cisplatin. This may in turn inhibit the phosphorylation of p90RSKs and ERK, thus restoring important apoptotic mechanisms in tumor cells. Although p90RSKs and HSP27 are co-expressed and co-localized in untreated SKOV-3 cells, the combination of cisplatin and melatonin suppressed their co-localization, documenting that melatonin may induce apoptosis via inhibition of the ERK/p90RSKs/HSP27 anti-apoptotic pathway (65). In SKOV-3 cells, the combination of cisplatin with melatonin induced caspase-3 activation and cleavage of poly-(ADP-ribose) polymerase (PARP), increasing the cisplatin-induced apoptosis together with elevated levels of MKP3, a specific phosphatase that controls MAPK activation (65). We recently verified the effect of long-term melatonin therapy on the pro-apoptotic (p53, BAX, total and cleaved caspase-3) and anti-apoptotic (Bcl-2 and survivin) proteins in an *in vivo* model of OC. Melatonin promoted upregulation of p53, BAX and activated caspase-3 followed by DNA fragmentation as observed by TUNEL-positive nuclei. Although Bcl-2 was unaltered, the survivin levels were reduced after melatonin therapy (71), identifying one of the possible routes by which melatonin causes OC cell apoptosis (Figure 2).

A recent publication revealed that exogenous melatonin treatment for 60 days efficiently reduced OC mass and the incidence of malignant tumors (e.g. high-grade serous papillary, sarcoma and undifferentiated carcinoma) in an ethanol-preferring rat model. These OCs also exhibited soft and mobile tissues with no peritoneal adhesions (72). To investigate whether melatonin activates human epidermal growth factor receptor (HER) and its downstream targets, the two major subtypes that occur in many cancers, termed HER-2 and HER-4, were studied. In OC cells, HER-2 and HER-4-mediated signaling pathways are strongly related to tumor survival, progression and metastasis. Notably, intraperitoneal administration of long-term melatonin (200 µg/100 body weight/day) efficiently suppressed the OC-related increase in the levels of HER-2, protein kinase B (phospho-Akt), mammalian target of rapamycin (mTOR) and p38 mitogen-activated protein kinase (p38 MAPK) (73), thus demonstrating its probably potential as an adjuvant therapy (Figure 2).

Toll-like receptors (TLRs) are a family of active molecules involved in host defense. Emerging evidence suggests TLRs are associated with cancer development in the context of microbial infection, inflammation, injury and tissue repair (74). They are expressed on the surface of OC cells, where especially the TLR2 and TLR4 are associated with chemoresistance to drugs and poor prognosis. Interestingly, melatonin treatment for 60 days induced downregulation of TLR4 and its downstream molecules including myeloid differentiation factor 88 (MyD88), TLR-associated activator of interferon (TRIF), nuclear factor-kappa B (NF-κB p65), inhibitor of NF-κB alpha (IkBα), IkB kinase alpha (IKK-α), TNF receptor-associated factor 6 (TRAF6), interferon regulatory factor 3 (IRF3), interferon β (IFN-β), tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) (2). Melatonin therapy attenuated the inflammatory response in OC cells through inhibition of either canonical or non-canonical signaling pathways, which may reduce the resistance to the effects of other drugs (Figure 2).

Angiogenesis is essential for OC progression and metastasis and molecules with anti-angiogenic capacities typically reduce tumor growth. Hypoxia-inducible factor 1 (HIF-1) is considered a poor prognostic factor and a promising target for the treatment of OC (71). There is considerable evidence showing that melatonin treatment significantly reduces the activity and expression of HIF-1α in both *in vitro* and *in vivo* tumors (71,75). In xenograft model of breast cancer, melatonin significantly reduced vascular endothelial growth factor receptor 2 (VEGFR2) together with a reduction in micro-vessel density (76). Similarly, our recent results showed that long-term administration of melatonin downregulated VEGF, TGFβ-1, VEGFR2 and HIF-1α in an animal model of DMBA-induced OC, in addition to having an important effect against neovasculogenesis (Figure 2). These anti-angiogenic effects seem to involve MT1 activation (77).

In a recent study, we used quantitative proteomic analysis to obtain new insights into the broad cellular effects of melatonin in an *in vivo* model of OC. Although no apparent histopathological changes were observed in the serous papillary architecture, melatonin downregulated proteins involved in diverse metabolic systems, including those associated with generation of metabolites and cell energy, HIF-1 signaling, endoplasmic reticulum stress-associated pathways, antigen processing and presentation, and cancer-related proteoglycans. The downregulated proteins related to mitochondrial processes and include glyceraldehyde-3-phosphate dehydrogenase, fructose-bisphosphate aldolase A, pyruvate kinase isozymes M1/M2, malate dehydrogenase, L-lactate dehydrogenase (LDH) A chain, protein disulfide isomerase (PDI) A3 and A6, creatine kinase B, ATP synthase subunit α, 78-kDa glucose-regulated protein (GRP78) and peptidyl-prolyl *cis*-trans isomerase A. These metabolic alterations may markedly affect aerobic glycolysis (Warburg effect) resulting in low proliferation and reduced metastatic potential of the cells. A few molecules were overexpressed and include ATP synthase subunit β, fatty acid-binding protein and 10-kDa heat-shock protein (78).

Melatonin has beneficial effects in combination with OC therapies

In 2000, a brief investigation by Bartsch *et al.* (79) reported that melatonin (varying from 10^{-6} to 10^{-10} M) and pineal extract YC05R (500 and 1000 Da) inhibited the growth of cultured primary OC cell obtained from patients with advanced disease. The physiological concentration of melatonin (10^{-9} M) was even more potent than cisplatin in terms of sensitivity and cell growth.

Melatonin is commonly used to quell the adverse effects of concurrent chemotherapy. An interesting paper by Jang *et al.* (80) described the protective effect of melatonin on the ovarian follicles of cisplatin-induced mouse ovaries. Besides preventing the disruption of follicular reserve via suppression of phosphorylation of PTEN/AKT/FOXO3a signaling pathway, melatonin (at dose of 15 or 30 mg/kg for 3 days) reduced the adverse effects of cisplatin. Thus, melatonin is indicated for use in combination with chemotherapy for young female cancer patients. Of great significance, platinum- and taxol-based chemotherapeutic agents for OC work as a first approach, but the tumors often become resistant to chemotherapy. The combination of chemotherapeutic agents with low cytotoxicity along with photodynamic therapy (PDT) can be applied as a promising strategy to overcome this problem. Melatonin enhances the generation of ROS at pharmacological concentrations in cancer cells in contrast to its well-known free radical-scavenging actions in normal cells (81). The combination of melatonin and PDT in SKOV-3 cells leads to a high production of ROS, and the induction free-radical

mediated apoptosis of tumor cells (82). In addition to its well-recognized oncostatic effect, co-administration of melatonin decreases anxiety-like symptoms, depression and general toxicity arising from chemotherapy (83,84).

Concluding remarks

Searching for molecules that substantially alter these important signaling pathways may be relevant to the treatment of OC. In this perspective, melatonin has added significant contributions to improve functional outcomes in *in vitro* and *in vivo* studies, demonstrating its ability to act as combination adjuvant in other therapeutic opportunities for OC. On the basis of why melatonin should be used for cancer treatments, it seems possible that melatonin acts killing damaged cells while promoting survival of injured normal cells. Considering that current clinical treatments are sometimes limited and there is no consistent evidence for efficacy in reducing stable solid tumors following chemotherapy alone, we strongly encourage consideration of the associated use of melatonin in the oncotherapeutic possibilities for OC. The potential of melatonin as a natural molecule also supports its efficacy in reducing adverse effects arising from conventional OC treatments. Although this important role has long been known, it has not been properly exploited at the clinical level. Considering that melatonin is an endogenously produced molecule with no toxicity and is available at pharmaceutical grade purity for humans, the use of melatonin may be helpful in the treatment of OC.

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References

1. Reiter, R.J. et al. (2016) Melatonin as an antioxidant: under promises but over delivers. J. Pineal Res., 61, 253–278.
2. Chuffa, L.G. et al. (2015) Melatonin attenuates the TLR4-mediated inflammatory response through MyD88- and TRIF-dependent signaling pathways in an in vivo model of ovarian cancer. BMC Cancer, 15, 34.
3. Blask, D.E. et al. (2002) Melatonin as a chronobiotic/anticancer agent: cellular, biochemical, and molecular mechanisms of action and their implications for circadian-based cancer therapy. Curr. Top. Med. Chem., 2, 113–132.
4. Su, S.C. et al. (2017) Cancer metastasis: mechanisms of inhibition by melatonin. J. Pineal Res., 62, e12370.
5. Reiter, R.J. et al. (2017) Melatonin, a full service anti-cancer agent: inhibition of initiation, progression and metastasis. Int. J. Mol. Sci., 18, E843.
6. Martin, T.A. et al. (2009) Loss of tight junction barrier function and its role in cancer metastasis. Biochim. Biophys. Acta, 1788, 872–891.
7. Hanahan, D. et al. (2011) Hallmarks of cancer: the next generation. Cell, 144, 646–674.
8. Nooshinfar, E. et al. (2017) Melatonin, an inhibitory agent in breast cancer. Breast Cancer, 24, 42–51.
9. Wu, S.M. et al. (2016) Melatonin set out to ER stress signaling thwarts epithelial mesenchymal transition and peritoneal dissemination via calpain-mediated C/EBPβ and NFκB cleavage. J. Pineal Res., 60, 142–154.
10. Zhou, Q. et al. (2014) Melatonin inhibits the migration of human lung adenocarcinoma A549 cell lines involving JNK/MAPK pathway. PLoS One, 9, e101132.
11. Xu, C.S. et al. (2015) Involvement of ROS-alpha v beta 3 integrin-FAK/Pyk2 in the inhibitory effect of melatonin on U251 glioma cell migration and invasion under hypoxia. J. Transl. Med., 13, 95.
12. Cos, S. et al. (1998) Influence of melatonin on invasive and metastatic properties of MCF-7 human breast cancer cells. Cancer Res., 58, 4383–4390.
13. Canel, M. et al. (2013) E-cadherin-integrin crosstalk in cancer invasion and metastasis. J. Cell Sci., 126 (Pt 2), 393–401.
14. Ortíz-López, L. et al. (2009) ROCK-regulated cytoskeletal dynamics participate in the inhibitory effect of melatonin on cancer cell migration. J. Pineal Res., 46, 15–21.
15. Borin, T.F. et al. (2016) Melatonin decreases breast cancer metastasis by modulating Rho-associated kinase protein-1 expression. J. Pineal Res., 60, 3–15.
16. Rudra, D.S. et al. (2013) Melatonin inhibits matrix metalloproteinase-9 activity by binding to its active site. J. Pineal Res., 54, 398–405.
17. Lin, Y.W. et al. (2016) Melatonin inhibits MMP-9 transactivation and renal cell carcinoma metastasis by suppressing Akt-MAPKs pathway and NF-κB DNA-binding activity. J. Pineal Res., 60, 277–290.
18. Lamouille, S. et al. (2014) Molecular mechanisms of epithelial-mesenchymal transition. Nat. Rev. Mol. Cell Biol., 15, 178–196.
19. Min, C. et al. (2008) NF-kappaB and epithelial to mesenchymal transition of cancer. J. Cell. Biochem., 104, 733–744.
20. Chua, H.L. et al. (2007) NF-kappaB represses E-cadherin expression and enhances epithelial to mesenchymal transition of mammary epithelial cells: potential involvement of ZEB-1 and ZEB-2. Oncogene, 26, 711–724.
21. Wang, X. et al. (2007) Oestrogen signalling inhibits invasive phenotype by repressing RelB and its target BCL2. Nat. Cell Biol., 9, 470–478.
22. Goncalves, N.N. et al. (2014) Molecular markers of angiogenesis and metastasis in lines of oral carcinoma after treatment with melatonin. Anticancer Agents Med. Chem., 14, 1302–1311.
23. Mao, L. et al. (2012) Circadian gating of epithelial-to-mesenchymal transition in breast cancer cells via melatonin-regulation of GSK3β. Mol. Endocrinol., 26, 1808–1820.
24. Pariente, R. et al. (2016) Melatonin sensitizes human cervical cancer HeLa cells to cisplatin-induced cytotoxicity and apoptosis: effects on oxidative stress and DNA fragmentation. J. Pineal Res., 60, 55–64.
25. Gao, Y. et al. (2017) Melatonin synergizes the chemotherapeutic effect of 5-fluorouracil in colon cancer by suppressing PI3K/AKT and NF-κB/iNOS signaling pathways. J. Pineal Res., 62, e12380.
26. Mayo, J.C. et al. (2017) IGFBP3 and MAPK/ERK signaling mediates melatonin-induced antitumor activity in prostate cancer. J. Pineal Res., 62, e12373.
27. Quintana, C. et al. (2016) Melatonin enhances hyperthermia-induced apoptotic cell death in human leukemia cells. J. Pineal Res., 61, 381–395.
28. Codenotti, S. et al. (2015) Melatonin decreases cell proliferation, impairs myogenic differentiation and triggers apoptotic cell death in rhabdomyosarcoma cell lines. Oncol. Rep., 34, 279–287.
29. Li, W. et al. (2017) Melatonin treatment induces apoptosis through regulating the nuclear factor-κB and mitogen-activated protein kinase signaling pathways in human gastric cancer SGC7901 cells. Oncol. Lett., 13, 2737–2744.
30. Li, W. et al. (2016) 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., 36, 2861–2867.
31. Nooshinfar, E. et al. (2016) Melatonin promotes ATO-induced apoptosis in MCF-7 cells: proposing novel therapeutic potential for breast cancer. Biomed. Pharmacother., 83, 456–465.
32. Wei, J.Y. et al. (2015) Melatonin induces apoptosis of colorectal cancer cells through HDAC4 nuclear import mediated by CaMKII inactivation. J. Pineal Res., 58, 429–438.
33. Wang, R.X. et al. (2016) Melatonin downregulates nuclear receptor RZR/RORγ expression causing growth-inhibitory and anti-angiogenesis activity in human gastric cancer cells *in vitro* and *in vivo*. Oncol. Lett., 12, 897–903.
34. Colombo, J. et al. (2016) Effects of melatonin on HIF-1α and VEGF expression and on the invasive properties of hepatocarcinoma cells. Oncol. Lett., 12, 231–237.
35. González, A. et al. (2017) Melatonin inhibits angiogenesis in SH-SY5Y human neuroblastoma cells by downregulation of VEGF. Oncol. Rep., 37, 2433–2440.
36. Hardeland, R. (2014) Melatonin, noncoding RNAs, messenger RNA stability and epigenetics–evidence, hints, gaps and perspectives. Int. J. Mol. Sci., 15, 18221–18252.
37. Lee, S.E. et al. (2011) MicroRNA and gene expression analysis of melatonin-exposed human breast cancer cell lines indicating involvement of the anticancer effect. J. Pineal Res., 51, 345–352.

38. Mori, F. et al. (2016) Multitargeting activity of miR-24 inhibits long-term melatonin anticancer effects. Oncotarget, 7, 20532–20548.
39. Santoro, R. et al. (2013) Blockage of melatonin receptors impairs p53-mediated prevention of DNA damage accumulation. Carcinogenesis, 34, 1051–1061.
40. Siegel, R.L. et al. (2016) Cancer statistics, 2016. CA Cancer J. Clin., 66, 7–30.
41. Chuffa, L.G. et al. (2017) The role of sex hormones and steroid receptors on female reproductive cancers. Steroids, 118, 93–108.
42. Longacre, T.A. et al. (2014) WHO Classification of Tumours of Female Reproductive Organs. 4th edn. Lyon: IARC Press, pp. 11–86.
43. Prat, J. (2012) Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features. Virchows Arch., 460, 237–249.
44. Vergote, I. et al.; European Organization for Research and Treatment of Cancer-Gynaecological Cancer Group; NCIC Clinical Trials Group (2010) Neoadjuvant chemotherapy or primary surgery in stage IIIC or IV ovarian cancer. N. Engl. J. Med., 363, 943–953.
45. Cho, K.R. et al. (2009) Ovarian cancer. Annu. Rev. Pathol., 4, 287–313.
46. Ueland, FR. (2017) A perspective on ovarian cancer biomarkers: past, present and yet-to-come. Diagnostics, 7, 14.
47. Sapiezynski, J. et al. (2016) Precision targeted therapy of ovarian cancer. J. Control. Release, 243, 250–268.
48. Mungenast, F. et al. (2017) Clinical significance of the estrogen-modifying enzymes steroid sulfatase and estrogen sulfotransferase in epithelial ovarian cancer. Oncol. Lett., 13, 4047–4054.
49. Di Bella, G. et al. (2013) Melatonin anticancer effects: review. Int. J. Mol. Sci., 14, 2410–2430.
50. Tamura, H. et al. (2009) Melatonin and the ovary: physiological and pathophysiological implications. Fertil. Steril., 92, 328–343.
51. Chuffa, L.G. et al. (2011) Long-term melatonin treatment reduces ovarian mass and enhances tissue antioxidant defenses during ovulation in the rat. Braz. J. Med. Biol. Res., 44, 217–223.
52. Chuffa, L.G. et al. (2011) Long-term exogenous melatonin treatment modulates overall feed efficiency and protects ovarian tissue against injuries caused by ethanol-induced oxidative stress in adult UChB rats. Alcohol. Clin. Exp. Res., 35, 1498–1508.
53. Tamura, H. et al. (2013) Melatonin as a free radical scavenger in the ovarian follicle. Endocr. J., 60, 1–13.
54. Huang, H.S. et al. (2015) Mutagenic, surviving and tumorigenic effects of follicular fluid in the context of p53 loss: initiation of fimbria carcinogenesis. Carcinogenesis, 36, 1419–1428.
55. Zhao, M. et al. (2016) The reduction in circulating melatonin level may contribute to the pathogenesis of ovarian cancer: a retrospective study. J. Cancer, 7, 831–836.
56. Poole, E.M. et al. (2015) Urinary melatonin and risk of ovarian cancer. Cancer Causes Control, 26, 1501–1506.
57. Hill, S.M. et al. (2015) Melatonin: an inhibitor of breast cancer. Endocr. Relat. Cancer, 22, R183–R204.
58. Jablonska, K. et al. (2014) Expression of the MT1 melatonin receptor in ovarian cancer cells. Int. J. Mol. Sci., 15, 23074–23089.
59. Treeck, O. et al. (2006) Antiestrogens modulate MT1 melatonin receptor expression in breast and ovarian cancer cell lines. Oncol. Rep., 15, 231–235.
60. Liu, J. et al. (2016) MT1 and MT2 melatonin receptors: a therapeutic perspective. Annu. Rev. Pharmacol. Toxicol., 56, 361–383.
61. Ayoub, M.A. et al. (2004) Preferential formation of MT1/MT2 melatonin receptor heterodimers with distinct ligand interaction properties compared with MT2 homodimers. Mol. Pharmacol., 66, 312–321.
62. Pacini, N. et al. (2016) Oncostatic-cytoprotective effect of melatonin and other bioactive molecules: a common target in mitochondrial respiration. Int. J. Mol. Sci., 17, 341.
63. Petranka, J. et al. (1999) The oncostatic action of melatonin in an ovarian carcinoma cell line. J. Pineal Res., 26, 129–136.
64. Shen, C.J. et al. (2016) Melatonin suppresses the growth of ovarian cancer cell lines (OVCAR-429 and PA-1) and potentiates the effect of G1 arrest by targeting CDKs. Int. J. Mol. Sci., 17, 176.
65. Kim, J.H. et al. (2012) Melatonin synergistically enhances cisplatin-induced apoptosis via the dephosphorylation of ERK/p90 ribosomal S6 kinase/heat shock protein 27 in SK-OV-3 cells. J. Pineal Res., 52, 244–252.
66. Futagami, M. et al. (2001) Effects of melatonin on the proliferation and cis-diamminedichloroplatinum (CDDP) sensitivity of cultured human ovarian cancer cells. Gynecol. Oncol., 82, 544–549.
67. Akbarzadeh, M. et al. (2017) The impact of different extracellular matrices on melatonin effect in proliferation and stemness properties of ovarian cancer cells. Biomed. Pharmacother., 87, 288–295.
68. Tan, Y. et al. (1999) p90(RSK) blocks bad-mediated cell death via a protein kinase C-dependent pathway. J. Biol. Chem., 274, 34859–34867.
69. Clark, D.E. et al. (2005) The serine/threonine protein kinase, p90 ribosomal S6 kinase, is an important regulator of prostate cancer cell proliferation. Cancer Res., 65, 3108–3116.
70. Smith, J.A. et al. (2005) Identification of the first specific inhibitor of p90 ribosomal S6 kinase (RSK) reveals an unexpected role for RSK in cancer cell proliferation. Cancer Res., 65, 1027–1034.
71. Chuffa, L.G. et al. (2016) Apoptosis is triggered by melatonin in an *in vivo* model of ovarian carcinoma. Endocr. Relat. Cancer, 23, 65–76.
72. Chuffa, L.G. et al. (2013) Characterization of chemically induced ovarian carcinomas in an ethanol-preferring rat model: influence of long-term melatonin treatment. PLoS One, 8, e81676.
73. Ferreira, G.M. et al. (2014) Melatonin attenuates Her-2, p38 MAPK, p-AKT, and mTOR levels in ovarian carcinoma of ethanol-preferring rats. J. Cancer, 5, 728–735.
74. Rakoff-Nahoum, S. et al. (2009) Toll-like receptors and cancer. Nat. Rev., 9, 57–63.
75. Park, S.Y. et al. (2010) Melatonin suppresses tumor angiogenesis by inhibiting HIF-1alpha stabilization under hypoxia. J. Pineal Res., 48, 178–184.
76. Jardim-Perassi, B.V. et al. (2014) Effect of melatonin on tumor growth and angiogenesis in xenograft model of breast cancer. PLoS One, 9, e85311.
77. Zonta, Y.R. et al. (2017) Melatonin reduces angiogenesis in serous papillary ovarian carcinoma of ethanol-preferring rats. Int. J. Mol. Sci., 18, E763.
78. Chuffa, L.G. et al. (2016) Quantitative proteomic profiling reveals that diverse metabolic pathways are influenced by melatonin in an *in vivo* model of ovarian carcinoma. J. Proteome Res., 15, 3872–3882.
79. Bartsch, H. et al. (2000) Effect of melatonin and pineal extracts on human ovarian and mammary tumor cells in a chemosensitivity assay. Life Sci., 67, 2953–2960.
80. Jang, H. et al. (2016) Melatonin prevents cisplatin-induced primordial follicle loss via suppression of PTEN/AKT/FOXO3a pathway activation in the mouse ovary. J. Pineal Res., 60, 336–347.
81. Bizzarri, M. et al. (2013) Molecular mechanisms of the pro-apoptotic actions of melatonin in cancer: a review. Expert Opin. Ther. Targets, 17, 1483–1496.
82. Akbarzadeh, M. et al. (2016) Effects of combination of melatonin and laser irradiation on ovarian cancer cells and endothelial lineage viability. Lasers Med. Sci., 31, 1565–1572.
83. Lissoni, P. et al. (1995) Modulation of cancer endocrine therapy by melatonin: a phase II study of tamoxifen plus melatonin in metastatic breast cancer patients progressing under tamoxifen alone. Br. J. Cancer, 71, 854–856.
84. Lissoni, P. et al. (1999) Decreased toxicity and increased efficacy of cancer chemotherapy using the pineal hormone melatonin in metastatic solid tumour patients with poor clinical status. Eur. J. Cancer, 35, 1688–1692.