Review article

Does the use of melatonin overcome drug resistance in cancer chemotherapy?

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ABSTRACT

Our knowledge regarding the implications of melatonin in the therapy of numerous medical conditions, including cancer is constantly expanding. Melatonin can variably affect cancer pathology via targeting several key aspects of any neoplastic condition, including the very onset of carcinogenesis as well as tumor growth, differentiation, and dissemination. Numerous studies have examined the effects of melatonin in the context of various cancers reporting the enhanced efficacy of chemo/radiotherapy in combination with this compound. Reduced sensitivity and also resistance of cancer cells to antineoplastic agents are common events which might arise as a result of genomic instability of the malignant cells. Genetic mutations provide numerous mechanisms for these cells to resist cytotoxic therapies. Melatonin, due to its pleitropic effects, is able to correct these alterations in favour of sensitization to antineoplastic agents as evident by increased response to treatment via modulating the expression and phosphorylation status of drug targets, the reduced clearance of drugs by affecting their metabolism and transport within the body, decreased survival of malignant cells via altering DNA repair and telomerase activity, and enhanced responsiveness to cell death-associated mechanisms such as apoptosis and autophagy. These effects are presumably governed by melatonin's interventions in the main signal transduction pathways such as Akt and MAPK, independent of its antioxidant properties. Possessing such a signaling altering nature, melatonin can considerably affect the drug-resistance mechanisms employed by the malignant cells in breast, lung, hepatic, and colon cancers as well as different types of leukemia which are the subject of the current review.

1. Introduction

The human body encloses trillions of DNA carrying cells. The DNA instructs the cells to divide and differentiate. Under physiologic conditions, division and differentiation are organized and well-regulated processes; however, should unsolicited mutations strike the vital regions of the DNA encoding regulatory genes, the instructions of harnessed proliferation and differentiation become derailed, leading to an aberrant proliferation of cells, which ultimately gives rise to the development of cancer. In some cases, early detection enables surgical removal of tumors. However, other therapeutic measures such as radiotherapy, chemotherapy as well as novel targeted therapies are generally reserved for disseminating aggressive and metastatic cancers,

generally considered as the cornerstones of cancer therapy. Since the adoption of these therapeutic measures, they have evolved into curative options for some cancers. Unfortunately, however, two obstacles have impeded the achievement of a successful treatment, granting complete remission: (i) adverse toxicities in the healthy cells and tissues (off-target hits) and (ii) development of resistance to chemo/radiotherapy.

In general, the resistance of cancer cells can be categorized into intrinsic resistance and acquired resistance. Almost half of all cancer cases are resistant to chemotherapy per se, while the majority of the remaining acquires resistance at some point along the treatment course [1]. Genomic instability is a well-recognized trait of all cancer cells. This instability is typically consequent to damages to the DNA repair system, impaired checkpoints of DNA damage such as the p53 tumor

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suppressor gene, deflected cell cycle checkpoints, and increased loss of telomeres [2]. Keeping in mind that an individual tumor can approximately host 10^9–10^{12} cells with possibly 10^5 mutants, cultivation of a highly heterogeneous population within one tumor is expected to occur.

Variations in the patterns of mutations and epigenetic modifications in oncogenes, tumor suppressor genes and genes related to the development of drug resistance result in the expression of different resistance components and the down-regulation of elements conferring sensitivity to external insults [3]. With the presence of multiple clones within a tumor, cytotoxic therapies such as chemo/radiotherapy fabricate an evolutionary process of selection, favoring the survival of the fittest resistant clones and their rapid expansion [4]. To impede the propagation of resistant clones, a successful treatment must be capable of abating the resistance mechanisms as well as sensitizing the non-resistant cancer cells to cytotoxic therapy. Overcoming such a resistance can be achieved through the designing of novel multifunctional agents or by the co-administration of compounds with sensitizing functions. Undoubtedly, it is advantageous to select a compound with additional benefits such as (i) selective cytotoxicity on cancer cells, sparing the healthy cells and tissues, (ii) protection of healthy cells against chemo/radiotherapy toxicity, (iii) improving the patient's overall condition, and (iv) increasing the patient's quality of life and extending post-treatment survival. The extracted data from articles were included and summarized in Table 1.

2. Melatonin

In the late 1950s, Lerner et al. isolated *N*-acetyl-5-methoxy-tryptamine from the bovine pineal tissue. As *N*-acetyl-5-methoxy-tryptamine could trigger mela-nin aggregation within the skin melanocytes, and resembled the structure of sero-tonin, the compound was labeled as "melatonin" [5]. Melatonin holds evolutionary ties to the most primitive living forms, the cyanobacteria, where it presumably acted as an essential radical scavenger, thus assisting in the survival of the organism by quenching the immense free radical content of the cyanobacteria [6]. As these bacteria, entered eukaryotic cells during endosymbiosis, their melatonin also participated in the functions of the eukaryotic cell host. During the course of time, melatonin's functions have indeed advanced, and at present, encompass complex signaling pathways and biological and hormonal functions [7].

3. Biosynthesis of melatonin

The pineal gland is an important site of melatonin production in vertebrates including humans. When a light signal is received by the retinal cells, it passes through the suprachiasmatic nucleus and then reaches the pineal gland where melatonin synthesis by the parenchymatous cells of the pineal gland is initiated with the conversion of tryptophan to 5-hydroxytryptophan and then to 5-hydroxytryptamine or serotonin, which undergoes acetylation by the enzyme aralkyl amine-*N*-acetyltransferase (AANAT) yielding *N*-acetylserotonin. Finally, *N*-acetylserotonin is methylated by the rate-limiting enzyme Acetylserotonin-*O*-methyltransferase (ASMT/HIOMT) [8], forming the final structure of melatonin. Melatonin is then released mainly into the cerebrospinal fluid to lower extents in the bloodstream [9]. The synthesis and release of pineal melatonin follow a circadian rhythm, controlled by the circadian pacemakers located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Likewise, the retina is an extra-pineal source of melatonin with circadian rhythmicity regulated by the retinal circadian clock [10]. There are other extra-pineal sources of melatonin including the immune cells, gastrointestinal (GI) tract, reproductive tract, skin and the lens [11]. Unlike the pineal melatonin which is released into the systemic circulation, the functions of extra-pineal melatonin are primarily localized [12]. An exceptional example is the GI melatonin. The GI tract is the leading organ possessing the highest rate of melatonin production and storage as it holds up to 400

times more melatonin than the pineal gland [13]. Under certain circumstances, this major extra-pineal source releases melatonin into the systemic circulation and also contributes to the daytime melatonin levels in both healthy and pinealectomized subjects [14].

4. Metabolism of melatonin

In humans, the primary site of melatonin metabolism is within the liver, where different isoenzymes of the cytochrome P_{450} (CYP450) mono-oxygenases such as CYP1A2, CYP1A1, and to a lesser extent CYP1B1, hydroxylate melatonin in the C6 position to form 6-OH-melatonin [12], the main metabolite of melatonin [15]. Subsequently, 6-OH-melatonin is conjugated mainly with sulfate and to some extent with glucuronide residues, and is then excreted in the urine [16]. Other less participating cytochromes such as CYP2C19 [15] demethylate melatonin into *N*-acetylserotonin [17]. In addition to the hepatic metabolism, non-enzymatic metabolism also occurs in all cells and also outside the cells via oxidation. Hydroxyl radical scavengers transform melatonin into cyclic-3-hydroxy melatonin (c3-OH-melatonin) [18]. In various tissues, especially the brain, metabolites such as *N*-acetyl-*N*-formyl-methoxy-kynuramine (AFMK) and a succeeding *N*-acetyl-5-methoxy-kynuramine can be generated by several reactions [19]. Additional metabolizing pathways include deacetylation by enzymes such as melatonin deacetylases or aryl acylamidases, generating 5-methoxytryptamine [12].

5. Receptors of melatonin

The means by which melatonin exerts its pleiotropic effects are diverse and comprise numerous signaling pathways and biochemical reactions. Melatonin engages both receptor-mediated and direct molecular interactions with targets ranging from free radicals to proteins and lipids involved in the modulation of cellular functions. Melatonin receptors consist of trans-membrane and nuclear binding proteins. Membrane receptors of melatonin, MT_1, and MT_2, belong to G-protein coupled receptors (GPCR) superfamily carrying seven transmembrane α-helix domains [20]. The activation of MT receptors typically leads to G_i-mediated reduction of cAMP and the subsequent decrease in PKA/CREB signaling. MT_1 receptors via activating G_{q11} increase the cytoplasmic calcium content. MT_2 receptors can inhibit the formation of cGMP, while both MT receptors can stimulate the activity of protein kinase C (PKC) by stimulating PLC-β which in turn involves MAPK/MEK/ERK and also PI3K/Akt signaling [16]. $MT_{1/2}$ receptors may play roles in some of the antitumor actions of melatonin, as they have been demonstrated to inhibit the uptake of linoleic acid, an essential promoter of tumor growth and progression in hepatoma cells [21]. Cytoplasmic binding partners of melatonin include proteins such as the enzyme quinone oxidoreductase 2, formerly acknowledged as the MT_3 receptor [22], as well as calmodulin (CaM), tubulin, and calreticulin [7]. Nuclear receptors of melatonin belong to the retinoic acid-related orphan receptors (ROR) family including RORα1, RORα2 and RZRβ subtypes [23]. Nuclear receptors of melatonin are also involved in gene regulation. For instance, RORα binding can upregulate the expression of HIF-1α, an important regulatory protein in hypoxia and oxidative stress [24]. A crucial property of melatonin regarding its behavior towards malignant cells is its selective cytotoxicity against cancerous cells; for instance, under "normal conditions" melatonin activates proliferative ERK1/2 and Akt signaling in healthy cells. However, on the contrary cancer cells treated with melatonin suffer from the loss of ERK1/2 or Akt which impede their progression and potentially breaks their resistance to cytotoxic therapies. An interesting finding of a study regarding the effects of melatonin co-treatment with doxorubicin on resistant cancer cells [25], which indicates the conditional nature of melatonin's actions and its selective cytotoxicity towards cancerous cells, was that the suppression of ERK1/2 and AKT by melatonin, as seen in MCF-7 tumor cells, was not observed in cardiomyocytes of the

Table 1
The effects of melatonin on drug resistance mechanisms in various cancer.

Type of cancer	Model/cell lines	Therapeutic agent	Dose of melatonin	Effects of the combination	Additional observations	Reference
Alteration of drug receptors						
NCSL	H1975^{T790M}, HCC827	Gefitinib	1 mM	Synergistic cell death Deactivation of the mutated receptor	↓p-EGFR ↓Akt ↓Anti-apoptotic proteins	[88]
Breast	MCF-7$^{ERα+}$ xenograft in athymic nude rat	Tamoxifen	2.5 µg/day	Enhanced tumor regression Reduced ERα phosphorylation	↓p-ERα ↑Tumor cAMP ↓LA uptake and 13-HODE production ↓Warburg effect ↑Apoptosis ↑Autophagy ↓p-ERK1/2, p-Akt, p-SRC, p-FAK, p-STAT3, p-CREB, and NFκB/p65	[84]
Enhanced biotransformation and efflux of drugs						
Breast	MCF-7$^{ERα+}$ xenograft in athymic nude rat	Doxorubicin	2.5 µg/day	Enhanced tumor regression Reduced expression of metabolizing enzymes CBR1, and AKR1C3 Reduced expression of BCRP	↓Tumor cAMP ↓LA uptake and 13-HODE production ↓Warburg effect ↓p-ERK1/2, Akt, NFκB, PKCα, PKCδ, p-PDK1, p-RSK2, p-STAT3, CREB, HER2, HER3, PCNA	[25]
Breast, colon, mouse leukemia	HBL-100, MCF-7, LoVo, P388 plus their ADR types P388, and P388 ADR mice xenograft	Doxorubicin	200–1000 pg/mL	Synergistic cell death Significant increase in intracellular doxorubicin concentration	↑Survival time of mice bearing P388/ADR	[97]
Colon	LoVo, LoVo resistant to doxorubicin	Doxorubicin	0.1 mM, and 1 mM	Synergistic cell death (1 mM) Decreased P-gp (0.1 mM) only in a certain group Increased P-gp (1 mM)	Reduction of P-gp is dose-dependent Doxorubicin cytotoxicity is increased	[98]
Brain tumor stem cells, and glioma	NSC23, NSC7-2, NSC11, and A172	Temozolomide, doxorubicin, mitoxantrone	1 mM	Enhanced cell toxicity Decreased expression of BCRP but not MDR1 or MRP1 Increased gene expression of P-gp (ABCB1)	Increased intracellular concentration of mitoxantrone Increased methylation of BCRP gene promoter	[96]
DNA damage repair						
Breast	MCF-7	X irradiation	1 nM, 10 µM, 1 mM	Enhanced cell death (max. effect by 1nM) Enhanced decrease in DNA-PKc, and RAD51 (max. effect by 1 nM)	G$_2$-M phase arrest, with higher G$_0$-G$_1$, and lower S phase cell percentage	[106]
Apoptotic cell death						
Renal, breast, glioma	Caki, MDA231, U87-MG	Kahweol	1 mM	Synergistic enhancement of apoptosis P53-independent upregulation of PUMA via CHOP induction Effects on apoptosis were independent of redox capacities	↑PUMA ↑DEVDase, PARP cleavage	[116]
NSCL	H1299, A549	berberine	1 mM	Additive enhancement of apoptosis	↓p-Akt, p-ERK ↓NFκB, COX-2 ↓AP-2β, hTERT	[124]
Colon	LoVo, SW480	Ursolic acid	1 mM	enhancement of apoptosis Increased translocation of NFκB and p300 to cytoplasm and reduced binding to the COX-2 promoter	↓Cell migration ↓MMP9, CDH1	[122]
Human leukemia	HL-60	Puromycin	1 mM	Enhancement of apoptosis	↑Sub-G$_1$, cell percentage, ↓G$_2$-M arrest ↑AMPK activation ↓DNA repair enzymes chk1/2	[125]

(continued on next page)

Table 1 (continued)

Type of cancer	Model/cell lines	Therapeutic agent	Dose of melatonin	Effects of the combination	Additional observations	Reference
Hepatoma	HepG2, Hep3b2.1–7, SNU-449, Bel-4702	Cisplatin	1 mM	Enhancement of apoptosis ↓p-IKKα/β, suppressed the nuclear translocation of NFκB, and reduced binding to COX-2 promoter	Enhancement of migration Inhibition ↓Bcl-2 ↓AP-2β/hTERT	[121]
Esophageal	KYSE520, KYSE410, KYSE150, KYSE30, Eca109, Eca18, and NE1, NE3 non-cancerous epithelial cells, Eca109 xenograft in BALB/c mice	5-Fluorouracil	5 mM	Enhancement of apoptosis ↑Caspase-8, -3, -7 ↓MEK/Erk ↓GSK3β/Akt	Melatonin alone selectively reduced growth, migration, and apoptosis Significant reduction of tumor growth in-vivo	[126]
Breast	MCF-7	Arsenic trioxide	1 nM	Synergistic enhancement of apoptosis, ↑p-53 activity, and p21 ↑Bax/Bcl-2 ↓Survivin	↓c-Myc, ↓hTERT	[123]
Breast	SK-BR-3 MDA-MB-231 MCF-10A normal breast cells	Arsenic trioxide	2 mM	Synergistic enhancement of cell death Cell death dependent on ROS ↑Redd1 ↓Akt, ↓mTOR, p70-S6K signaling ↑p38, and JNK	Cell death was dependent on mTOR inhibition, and oxidative stress	[127]
hepatoma	HepG2, Bel-7402	Doxorubicin	0.01 mM	Synergistic enhancement of cell death	↓Bcl-2 ↑Bax, caspase-3	[115]
Ewing's sarcoma	SK-N-MC	Vincristine Ifosfamide	1 mM	Synergistic enhancement of cell death ↑Caspase-3, -7, -9, and Bid	MAPK, and PKB/Akt not involved in apoptosis	[129]
Ovarian	SK-OV-3	Cisplatin	2 mM	Synergistic enhancement of cell death ↑MKP3 ↓ERK ↓p90RSK ↓HSP27	ERK inhibition due to increased MKP3	
Hepatoma	HepG2, SMMC-7721	Doxorubicin (± tunicamycin)	1 mM	Enhancement of apoptosis, and reduction of resistance due to ER-stress ↓Akt, ↓Survivin ↑CHOP	↓ER-stress upregulation of Akt and promotion of apoptosis resistance	
Breast	MDA-MB-231	Puromycin	3 mM	Synergistic increase of cell death ↓45S pre-rRNA ↑UBF, XPO1, IPO7	↓Akt, mTOR, STAT3 ↓Bcl-xL, Mcl-1, cyclin D1, and cyclin E	[153]
Glioma	A172, U87	TRAIL	1 mM	↑TRAIL-induced apoptosis ↑DR5 ↓Bcl-2, and survivin	↓PKC/Akt	[131]
NSCL	A549	TRAIL	0.5 mM	↑TRAIL-induced apoptosis ↓Bcl-2, and Bcl-xL	↓HIF-1α, and PHD2 ↓Akt Reduction of hypoxia mediated apoptosis resistance	[133]
Prostate	LNCaP, PC3	TNF-α, TRAIL, doxorubicin, docetaxel, etoposide	1 mM	↑Differentiation into neuroendocrine phenotype ↑TRAIL, and TNF-induced apoptosis in androgen dependent LNCaP	↑Apoptosis due to differentiation	[135]
Autophagy/mitophagy						
Hepatoma	HepG2, HuH7, Hep3B	Sorafenib	1 mM	↑Mitophagy ↑PINK1, and PARKIN	↑ROS ↓Bax	[148]

same animals, and this effect along with further upregulation of SOD2 expression could protect cardiomyocytes from doxorubicin-induced toxicity [26]. A preliminary explanation for this phenomenon would be the G-protein switching. A process during which $MT_{1/2,}$ instead of their typical coupling with G_i (when activated in cancerous cells), preferably couple to Gs and finally creates opposing effects in healthy and cancerous cells. This phenomenon has already been documented for a few GPCRs notably Calcium-sensing receptor (CaR) which is also a GPCR normally coupled to Gi. The result of CaR activation in normal cells (mammary epithelial cells) is the inhibition of the secretion of a specific protein (SP). On the contrary, when CaR is activated on immortalized or cancerous cells, the secretion of SP is stimulated. Follow-up experiments revealed that CaR couples with Gi in normal cells, while it couples with Gs in cancerous cells [27]. This hypothesis and the possibility that MT receptors couple with other G proteins should be further evaluated as it is crucial for proceeding our understanding of the paradoxical effects of melatonin in healthy vs. cancer cells.

6. Antioxidant capacities of melatonin

Melatonin, along with its primary metabolites, AFMK and AMK, possess strong antioxidant capabilities. The mechanisms of antioxidant actions of melatonin are diverse and include direct scavenging of free radicals such as ROS and RNS, upregulation of cellular antioxidant defence and the inhibition of pro-oxidant enzymes, reduction of free radical formation by protecting the mitochondria, chelation of Fe^{2+}/Fe^{3+} ions and the subsequent reduction of harmful products of Fenton reaction [28]. Perhaps the most remarkable trait of melatonin regarding its effects on oxidative stress is the conditional nature of melatonin antioxidant activities in a manner that under certain circumstances like malignancies, melatonin can paradoxically act as a pro-oxidant, inducing oxidative burden on the cells and promoting their death by apoptosis [29].

7. Melatonin in health and disease

Initially, melatonin was thought of as a mere hormone involved in the regulation of the circadian and circannual cycles [30]. However, the apparent omnipresence of melatonin and the relevant receptors [31] along with their respective biochemical and cellular functions, promoted the status of melatonin from a usual brain hormone to a pleiotropic regulatory molecule with potent antioxidant [32] and anti-inflammatory [33] effects and involved in DNA repair regulation [34], regulation of cardiovascular and nervous system functions [35,36], modulation of the immune system [37,38], senescence [39], metabolic functions, energy balance [31], anti-cancer [40,41] and cytoprotective [42] functions that sustains cell survival by actively participating in the neutralization of oxidative damage [43].

Considering the substantial involvement of melatonin in many fundamental biological functions, it is anticipated that this molecule could have beneficial effects in a diverse spectra of disorders. Indeed a vast number of studies dedicated to the treatment of diseases with melatonin have consistently obtained promising results. In the central nervous system, melatonin has been used in treating conditions such as insomnia [44,45], Alzheimer's disease [46], Parkinson's disease [47], Huntington's disease [48], Amyotrophic lateral sclerosis [49], migraine and other disorders with headache manifestations [50], gastrointestinal diseases such as gastro-esophageal reflux disease (GERD), peptic ulcer [51,52], irritable bowel syndrome [53], inflammatory bowel disease [54], and also pancreatitis [55], which have been variably attenuated by melatonin. Moreover, the efficacy of melatonin treatment is well-evidenced in metabolic disorders such as obesity and diabetes complications [16] and in cardiovascular disorders notably hypertension, myocardial infarction, and coronary heart diseases [56]. Finally, exploiting melatonin's potent antioxidant and cytoprotective properties has yielded into exceptional results encompassing protection from

various toxicants such as prescription drugs [57,58], pesticides [59], poisonous metals [60–62], and numerous other toxins [63].

8. Melatonin and cancer

The earliest evidence of the possibility of associations between melatonin and cancer were provided by the observations of disturbed melatonin secretion in cancer patients [64] and increased tumorogenesis in pinealectomized animals which were attenuated by exogenous melatonin administration [65]. Further studies exposed the influence of night shift work on the higher incidence of cancers such as breast [66], colorectal [67], endometrial [68] and several others such as ovarian, prostate, lung, and also gastric cancer [69]. Such observations, along with findings of lower breast cancer incidence among the blind or visually impaired women [70,71] or in those with higher urinary melatonin levels [72] connoted melatonin as a key participant in the etiology and pathology of cancers [73]. The compelling results of recent studies prompted the International Agency for Research on Cancer to recognize shift works involving disruption of circadian rhythms as a probable carcinogen to humans [74].

Melatonin not only reduces the events leading to the formation of cancer, but also displays powerful oncostatic capabilities via its effects on cell cycle, apoptosis, oxidative stress, immunostimulation, and growth signals [40]. These effects can be translated into the ability to impede the formation, growth, angiogenesis, dedifferentiation, and metastasis of malignancies such as gastric cancer [75], breast cancer [76], ovarian cancer [77,78], pancreatic cancer [79,80], colorectal cancer [81], and prostate cancer [41,82,83]. In addition to the aforementioned carcinogenic effects of disrupted circadian and melatonin rhythms, intriguing results have been obtained from recent studies experimenting on the disruption of rhythms using dim light exposure at night (dLEN). The rationale for using dim light as opposed to bright light is that dLEN only interferes with melatonin function, while the normal SCN-driven behaviors such as circadian feeding and drinking persist [84]. In an in vivo study on rats bearing estrogen receptor ($ER\alpha^+$) MCF-7 breast cancer xenograft tumors, breaking the normal light/dark cycle with dLEN increased the tumor development rate, metabolic activity, and growth rate more than two folds. However, most interestingly it granted an almost complete intrinsic resistance to tamoxifen therapy.

9. Mechanisms participating in cancer resistance

A variety of mechanisms collaborate in conferring resistance to cytotoxic therapies; resistance could either be limited to a particular class of drug or be developed against chemically dissimilar and functionally unrelated drugs simultaneously, a phenomenon recognized as multidrug resistance [85]. The best-known examples of such mechanisms include: (i) alterations in the drug target, (ii) enhanced rate of drug efflux from cells, (iii) elevated drug biotransformation, (iv) increased DNA damage repair, (v) reduced sensitivity to apoptosis and overactive pro-survival signaling, (vii) dedifferentiation and epithelial-mesenchymal transition (EMT), and (viii) distorted autophagy machinery [86]. It is remarkable that a combination of these mechanisms can simultaneously occur and in numerous occasions the underlying pathways involve more than one effect. Thus, the cellular system of resistance creates a complex nonlinear network, which requires a comprehensive view for its interpretation. Different mechanisms of drug resistance have been illustrated in Fig. 1.

9.1. Alterations in drug targets

The efficiency of a drug depends on a strong binding activity to its target. Alterations in the targets of the drug such as those induced by mutations or adjustments in their expression patterns could severely damper the efficacy of a chemotherapeutic agent. Furthermore, the
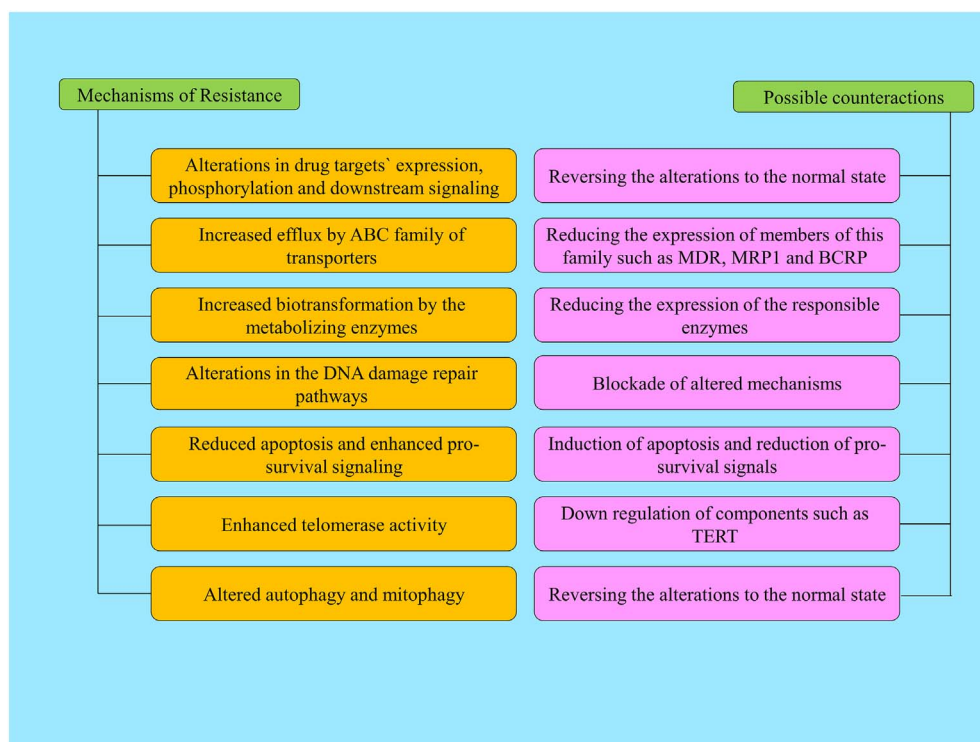
alterations could happen at the level of signal transduction pathways and negate the signaling streams necessary to produce the desired cytotoxic effect. These modalities occur in many cases in which tumors acquire resistance [86].

In perhaps one of the earliest studies on the cancer sensitizing effects of melatonin, pretreatment of MCF-7 breast cancer cells with melatonin (232 ng/mL) increased the potency of tamoxifen to inhibit breast cancer growth more than a hundred times [87]. In another in vivo study on rats bearing estrogen receptor (ERα+) MCF-7 breast cancer xenograft tumors [84], interrupting the normal light/dark cycle with dLEN granted an almost complete intrinsic resistance to tamoxifen therapy, as well as increased tumor development, metabolic activity, and growth rate. Rats subjected to dLEN had 24-h plasma melatonin levels dropped to almost undetectable levels and their tumors showed elevated estrogen receptor α (ERα) phosphorylation at Ser118 and Ser167 sites with higher levels of phospho-active ERK1/2, CREB, Akt, p-SRC, p-FAK, NF-κB/P65, and p-STAT3. However, these characteristics were abrogated in animals under normal light/dark rhythms (LD 12:12) and also in rats in the dLEN group supplemented with melatonin at night. A subsequent study obtained similar results regarding doxorubicin resistance [25]. Suppression of melatonin by dLEN induced the activity of key factors and signaling pathways involved in tumor growth and resistance such as EGFR members HER2 and HER3, and downstream effectors and pathways such as ERK1/2, PKA, CREB, AKT, and their regulator kinase, PAK-1, PDK-1, PKC α and δ, RSK2, NF-κB and STAT3 while restoring melatonin by supplementation suppressed these factors and overcame the resistance of MCF-7 tumors to doxorubicin.

Yun et al. performed a study regarding the effects of melatonin on drug-resistant NSCLC cells. Comparing a cell line with active EGFR responsive to the tyrosine kinase inhibitor (TKI) gefitinib, with cells harboring a point mutation (T790 M) which is responsible for modifying the ATP-binding pocket of EGFR receptors and consequent resistance to TKIs, they discovered that melatonin selectively reduced the phosphorylation of EGFR and downstream activation of Akt only in the cell lines bearing T790 M mutations, whereas gefitinib failed to produce this effect and could only inhibit the non-mutated EGFR. Furthermore, combining melatonin with gefitinib treatment resulted in a synergistic

inhibition of the cells with mutated EGFR and induced a twofold increase in the number of apoptotic cells, compared to the cells solely treated with gefitinib or the non-treated cells. These results demonstrate that in case of resistant conferring mutations such as T790 M in EGFR, which restrict the access of drug to the receptors, melatonin could re-establish treatment sensitivity by modulating phosphorylation and activation of receptors [88].

Changes in the expression of estrogen receptors have been associated with resistance to tamoxifen therapy. Furthermore, modulation of ERα, for instance via its phosphorylation at specific residues, reduces its affinity for tamoxifen and also reduces the DNA binding of tamoxifen-bound ERα [89]. Furthermore, it can overturn the effects of tamoxifen into agonist activities [90]. The activities of several kinases such as Akt, PKA, MAPK, SRC, and PAK-1 have been associated with tamoxifen resistance via modulation of ER expression or inducing ERα phosphorylation [91]. Also, overexpression of EGRF/HER2 and the activation of their downstream signaling pathways, which are associated with ER activity, can lead to increased cancer growth and resistance to therapy [92].

The ability of melatonin to modulate the expression of estrogen receptors in breast cancer tumors [93], as well as to mitigate the alterations in the estrogen receptors and the associated signaling pathways is an important aspect in counteracting resistance of tumors to chemotherapies.

9.2. Enhanced drug efflux

The majority of the antineoplastic drugs have intracellular targets. Hence, these drugs must be able to accumulate inside the cell and reach a sufficient concentration to be effective. Many resistant cancers exploit this liability by overexpressing members of the ATP-binding cassette (ABC) transporters. Numerous members of the ABC transporters are expressed in the plasma membranes of healthy cells and take part in the active transport of various compounds from the cell. However, enhanced efflux of drugs by members of ABC transporters, especially the P-glycoprotein (P-gp) also known as multidrug resistance protein 1 (MDR1) or ABCB1, multidrug resistance-associated protein 1 (MRP1),

and breast cancer resistance protein (BCRP), is one of the best-known mechanisms of cancer resistance. These members are overexpressed in various cancers such as gastrointestinal, lung and breast cancers, and cancers of hematopoietic origin [3,86]. Different strategies have been devised to overcome the resistance caused by ABC transporters including inhibition of the transporters by drugs such as the L-type calcium channel blocker verapamil or new generation compounds with high selectivity, depletion of ATP as a necessary substrate for their activity, and reduction of their expression by modulating underlying pathways such as the ERK pathway [94,95].

In MCF-7 breast tumor xenografts, exposure to dLEN increased the levels of BCRP which corresponded with the development of resistance to doxorubicin; however, in the group receiving melatonin supplementation concomitant with dLEN the levels of BCRP significantly decreased [25]. Another study using three brain tumor stem cell lines (NSC23, NSC7–2, and NCS11) and a malignant glioma cell line, A172, demonstrated that melatonin in combination with temozolomide, doxorubicin, or mitoxantrone can increase the toxicities of these drugs, synergistically. The brain tumor stem cells had higher expressions of BCRP and MDR1 than glioblastoma cells which explained their resistance to these three chemotherapeutics, but the expressions of MRP1 was similar in all cell lines. Interestingly, melatonin co-incubation decreased the mRNA levels and activity of BCRP, but not MDR1 or MRP1, by enhancing DNA methylation in the promoter region of BCRP gene; an observation consistent with the synergistic effects [96]. Granzotto et al. performed a study on the effects of different concentrations of melatonin ranging from 10 to 2000 pg/mL, alone or in combination with doxorubicin on MCF-7 breast adenocarcinoma, LoVo colon carcinoma, and P388 mouse leukemia cancer cell lines along with their anthracycline-resistant counterparts. In the MCF-7 cell line, a small but significant decrease in cell growth was observed at 10–100 pg/mL concentrations, while no concentrations of melatonin could enhance the cytotoxicity of doxorubicin. However, on the resistant MCF-7 cell line a modest growth inhibition was achieved by melatonin at the concentrations of 80 and 2000 pg/mL, while the cytotoxicity of doxorubicin was significantly increased following treatment with 100 pg/mL of melatonin. LoVo cell lines also showed growth inhibition after treatment with 100 pg/mL of melatonin in case of non-resistant cells and with 1000–2000 pg/mL in the case of resistant cells. Moreover, a slight but significant increase of doxorubicin cytotoxicity was observed at the concentration of 2000 pg/mL. Additionally, 40–80 pg/mL of melatonin reduced the growth of normal p388 leukemia cell lines with no enhancement of doxorubicin cytotoxicity at any concentrations tested, while their resistant counterparts, in addition to responding to 400–1000 pg/mL melatonin alone, displayed a pronounced dose-dependent enhancement of doxorubicin cytotoxicity by 100–200 pg/mL of melatonin. Furthermore, 10 ng/mL of melatonin increased the intracellular accumulation of doxorubicin in the resistant P388 cells. An in vivo investigation revealed that the addition of melatonin to doxorubicin in mice bearing the resistant P388 cell lines could increase their survival time compared with sole doxorubicin treatment. With the exception of P388 cell lines, the modifying effects of melatonin, although significant, were modest [97]. Consistent with these results, it was recently reported that melatonin enhanced the cytotoxicity of doxorubicin in regular and resistant LoVo cells expressing high levels of P-gp; the enhancements were less visible with higher concentrations of doxorubicin. Also, while the combination of doxorubicin and melatonin at a concentration of 0.1 mM reduced the percentage of resistant cells expressing P-gp, other combinations of 1 mM and 0.1 mM melatonin with varying doxorubicin concentrations increased P-gp expressing cells, in correlation with increased expression of ABCB1 gene, compared to doxorubicin alone [98]. It seems unlikely that melatonin contributed to the increased expression of P-gp and ABCB1 gene expression, as no such increase was seen when using melatonin alone. Moreover, considering the findings of other studies, it is reasonable to assume that doxorubicin is responsible for the increased expression of

P-gp. Nevertheless, more studies employing additional cell lines, other members of the ABC transporters, and combinations of various concentrations of melatonin, alone or with chemotherapeutic agents are required to fully elucidate the impact of melatonin on drug efflux systems. Moreover, considering that other mechanisms, such as post-translational modifications, could greatly influence the activity of the transport systems, the relative effects of melatonin at post-translational levels should also be clarified.

9.3. Biotransformation and detoxification of drugs

Metabolic activation or deactivation of antineoplastic agents, whether carried out by organs such as intestine, liver, and kidneys or inside the tumor cells by metabolizing enzymes and reactive nucleophiles, is crucial in determining the responsiveness or resistance of tumors to the therapy. Polymorphisms in genes involved in the biotransformation of antineoplastic drugs such as members of cytochrome P450 (CYP), uridine diphosphate glucuronosyltransferase (UGT), glutathione S-transferase (GST) significantly influence the clinical outcome of cancer therapies [99]. Equally important, tumors can utilize these biotransforming enzymes and also reactive nucleophiles such as glutathione to inactivate various drugs [100]. Examples of this include, deactivation of platinum compounds by high GSH levels in cancer cells [101], UGT1A1 inactivation of irinotecan, and detoxification of chemotherapeutics by GST, resulting in resistance to the cytotoxic compounds. Reduction of the GSH levels by pro-oxidant actions of melatonin in tumors has been reported [102]. Furthermore, the study by Xiang et al. discovered that the dLEN subjected MCF-7 breast tumor xenografts expressed higher levels of carbonyl reductase 1 (CBR1) and aldo-keto reductases (AKR) 1C1, 1C2, and 1C3. These reductases are reportedly implicated in acquired and intrinsic resistance [25]. Moreover, in case of doxorubicin, they increased the conversion of the active doxorubicin to the almost inactive doxorubicinol. Conversely, melatonin supplementation (~2.5 µg/day) suppressed the levels of CB1 and AKR1C3 [25]. A study found that melatonin could inhibit the expression of CYP450 members CYP11A and CYP17; this study should prompt more exhaustive experiments regarding the effects of melatonin on the expression profile of CYP members such as CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP2B6 that are involved in the biotransformation of antineoplastic agents [99]. Furthermore, Gradinaru et al. discovered that oxidative stress could significantly trigger the expression and activity of detoxifier enzymes such as UGT1A6 and 1A7 in rat cultured astrocytes as an adaptive response. This response, however, was prevented by melatonin, owing to its potent antioxidant activities [103]. Since a large number of chemotherapeutics heavily induce oxidative stress, it is possible that the same scenario could happen in the course of chemotherapy. In that case, co-treatment with the indoleamine could counteract the mentioned process, thus preventing the acquisition of resistance.

9.4. DNA damage repair systems

The antineoplastic action of the most chemotherapeutic agents depends on the DNA damage induction in rapidly proliferating cancer cells. The normal response to DNA damage is either activation of DNA repair systems or in extreme cases, death via apoptosis. Hence, the capacity of DNA repair in cancer cells is a major determinant of the treatment outcome of drugs that induce DNA damage. Evidently, dysfunctions in DNA repair mechanisms are common in cancers which lead to their reliance on an alternate repair pathway. Such a dependence provides an opportunity to obstruct the alternate repair system to specifically render the tumor incapable of DNA damage repair; this concept is termed "synthetic lethality" [104] [100]. At this susceptible status, the addition of a DNA damaging agent devastates the sensitized tumor.

The process of DNA damage repair includes detection of the injury

by the components of DNA damage response, activation of cell cycle checkpoints [105], and is based on the type of damage and the initiation of several repair methods such as nucleotide excision repair (NER), mismatch repair (MMR), base excision repair (BER), and in case of a double-strand break, homologous recombination (HR) and non-homologous end-joining (NHEJ).

The value of targeting DNA repair systems goes beyond the induction of synthetic lethality in tumors with repair deficiencies and is also useful in tumors with intact DNA repair systems since other participants such as regulators of the cell cycle can be provoked to induce DNA damage-induced apoptosis [105]. Therefore, a feasible strategy would be the reduction of DNA damage repair alongside the upregulation of a central cell cycle regulator, for instance, p53, tempting the cells to override cell cycle checkpoints which would sensitize the tumors to DNA damage-induced apoptosis and lead them to a mitotic catastrophe [105]. Adding a DNA damaging agent would be the final touch, as it would produce synergistic cytotoxicity.

Melatonin, which is a well-known enhancer of p53 in tumors, has been recently shown to enhance the reduction of RAD51 and DNA-PKcs, two members of double-strand DNA break repair and to decrease the G_2M phase arrest at its physiologic concentration (~1 nM), which results in sensitization of MCF-7 breast cancer cells to ionizing X irradiation as seen by significantly higher decrease of cancer cell proliferation [106].

There may exist other components participating in DNA damage repair. Reportedly, several classes of histone deacetylases (HDACs), including SIRT1 of the sirtuin family [107,108] are capable of promoting DNA repair. Hence, their inhibition by HDAC inhibitors has been suggested to be useful in chemosensitization [105]. Interestingly, melatonin possesses the ability to downregulate SIRT1 and this ability is in relevance with increased p53 and mitochondrial apoptosis in tumor cells [102]. In addition to the other consequences of SIRT inhibition, it can be presumed that the effects on DNA repair also assist in the antitumor capabilities of melatonin.

9.5. Cell death by apoptosis

Apoptotic cell death occurs with successive activation of various degrading enzymes [109]. The majority of antineoplastic agents, including irradiation, induce apoptosis in cancer cells. A brief example of this agents include the antimetabolites, DNA cross-linking agents, intercalating agents, protease inhibitors, topoisomerase 1/2 inhibitors, taxanes, vinca alkaloids and other agents [110]. This proves the significance of apoptosis in chemotherapy. This importance comes at a price as the resistance to apoptotic cell death has become the hallmark of cancers. Apoptosis resistance leads to the generation of tumors, their progression, and resistance to cancer therapies. As we review the process of apoptosis, it comes to mind that a large number of proteins and signaling transducers tightly regulate each step of apoptosis. However, this is a double-deck sword, as malignant cells can hamper the function of these regulators to evade death by apoptosis. Since exhaustive reviews have discussed this subject [111,112], we only provide a brief overview on some of the best-known mechanisms of cancer resistance to apoptosis. In general, resistance is acquired when the ratio of anti-apoptotic to pro-apoptotic factors increase; this can happen at numerous points. These mechanisms include: impaired expression and function of death receptors such as FasR and $DR_{4/5}$, deranged transport of TRAIL receptors ($DR_{4/5}$) to the cell surface, total loss of expression of TRAIL receptors, increased expression of decoy death receptors notably $DcR_{1/2}$ (it is worthy to note that these $DcR_{1/2}$ decoy receptors are not only dysfunctional in inducing apoptosis but could also activate NF-κB, opposing the entire apoptotic cell death) [113], increased c-FLIP expression, reduced caspase-8 expression and its phosphorylative deactivation by the Proto-oncogene tyrosine-protein kinase Src, increased expression of anti-apoptotic Bcl-2 family members, inactivation of pro-apoptotic Bcl-2 members, loss of Apaf-1, and eccentric expression of

IAPs most importantly XIAP and survivin.

The developing understanding of the mechanisms of apoptosis evasion and tumor resistance has made this subject an interesting field for the development of agents that can interfere with the resistance mechanisms and sensitize malignant cells to death. Judging by the numerous studies regarding the role of melatonin in the modulation of apoptosis, it has great potential to be used both in research and clinical setting. The following section is a review of latest studies regarding the sensitization of tumors to apoptotic cell death by melatonin.

Studies on numerous cell lines such as human keratinocytes, non-small cell lung cancer, laryngeal cancer, and hepatoma demonstrate the enhancement of doxorubicin cytotoxicity by melatonin via enhancing the apoptotic cell death [114]. Using HepG2 and Bel-7402 cell lines of hepatoma, Fan et al., observed a significant increase of doxorubicin-induced growth inhibition and apoptosis. Melatonin concentration of 0.01 mM produced the strongest synergy corresponding with reduced expression of Bcl-2, increased expression of Bax and a rise in activated caspase-3 level [115]. They further studied the combination of doxorubicin with melatonin using apoptosis-resistant HepG2 and SMMC-7721 hepatoma cell lines by inducing endoplasmic reticulum (ER) stress. The results suggested that melatonin increased the doxorubicin-induced apoptosis possibly via attenuation of ER stress-related AKT activation and consequently, reduced the expression of survivin, and increased the levels of CHOP, which is a transcription factor involved in mediating the ER stress-induced apoptosis and possibly in facilitating melatonin-induced cancer cells apoptosis through up-regulating PUMA [116]. Therefore, the normalization of elevated AKT activity re-sensitized the hepatoma cells to doxorubicin [117]. These results are in accordance with a study showing that melatonin alone could potentiate apoptosis in hepatoma cells under ER stress [118]. The results suggest that the down-regulation of COX-2 by melatonin was also involved in mediating increased CHOP levels and apoptosis, as administrations of celecoxib as a COX-2 inhibitor produced similar results. COX-2 is a well-known promoter of cancer and is suggested to confer the apoptosis resistance of cancers [119]. In fact, it has been shown that the down-regulation of COX-2 by melatonin, mainly by PI3K/AKT inhibition, is responsible for the enhancement of apoptosis seen in hepatocellular carcinoma cells through reducing IAP members survivin and XIAP; however, there were no changes in other IAP members cIAP-1/2 [120]. Moreover, these results were confirmed by observation of significant sensitization of four hepatocellular carcinomas (HepG2, Hep3B 2.1–7, SNU-449, and Bel-7402) to cisplatin by melatonin co-administration. The reduction of COX-2 and Bcl-2 and apoptosis enhancement as visualized by the release of Cyt-c and caspase-3 and -9, and PARP cleavage, was observed due to the suppression of phosphorylated IKKα/β, NF-κb P50/P65 nuclear translocation, and at last, the abrogation of P65 subunit binding to COX-2 promoter [121]. Additionally, a similar apoptosis enhancement by melatonin via the inhibition of NF-κB from binding to COX-2 promoter has been reported with the colon cancer cell lines LoVo and SW480 [122]. Induction of p53 might be the underlying cause of decreased survivin activity as it was declared in a case of synergistic apoptosis induction in breast cancer cells by melatonin and arsenic trioxide combination. Other downstream targets of p53 such as Myc and hTERT were also inhibited, which ultimately resulted in Bcl-2 upregulation and reduction of Bax [123].

In resistant NSCLC H1975 cells bearing a EGFR mutation, melatonin synergized the effect of gefitinib by the reduction of Akt phosphorylation, resulting in decreased expression of Bcl-2, Bcl-xL, and survivin, which in turn increased caspase-3 cleavage and doubled the number of apoptotic cells. However, the activity of p38 MAPK pathways remained unaffected [88]. In another study on NSCLC cell lines, melatonin potentiated the effects of a natural compound, berberine, on apoptosis by increasing Cyt-c release and caspase-9 cleavage, which involved the inhibition Akt and ERK1/2. These effects were translated into an enhanced tumor growth inhibition of lung cancer xenografts, in vivo [124]. Additionally, activation of AMPK signaling was reported in the

synergistic apoptosis of human leukemia cells by a melatonin-puromycin combination [125].

It is evident that the modulation of the principal signal transduction pathways by melatonin is pivotal in apoptosis induction. Inhibition of GSK3β/Akt and MEK/Erk pathways by melatonin has been demonstrated to enhance the cytotoxicity of 5-fluorouracil in esophageal carcinoma [126]. Furthermore, it has been reported that the addition of melatonin to arsenic trioxide can synergize the induction of apoptotic death in breast cancer cells via the up-regulation of Redd1, inhibition of mTOR/S6K and, increased P38/JNK signaling [127]. In Melatonin combination with cisplatin, the augmented inhibition of ERK and downstream p90RSK along with HSP27, two negative regulators of apoptosis, mediate their synergism in driving SK-OV-3 ovarian cancer cells towards apoptosis. On the contrary, melatonin combination with cisplatin attenuated cisplatin cytotoxicity in normal ovarian epithelial cells [128]. However, a study by Cascade-Zapico et al. reported that although melatonin produced synergistic apoptosis induction in combination with vincristine and ifosfamide on Ewing's carcinoma cells, neither any of the MAPK pathways including ERK, JNK, and p38her nor the PKB/Akt pathway were altered during this synergism. Still, melatonin co-treatment significantly increased ROS levels, followed by the activation of Bid and the caspases −3, −8, and −9, which implies the contribution of the extrinsic apoptosis pathway [129], which is plausible considering that melatonin induced the expression of Fas/FasL in Ewing's carcinoma cells [130]. In fact, several other studies employing cells lines of glioma, AML, ALL, CML, Burkitt's lymphoma, and NSCLC have also demonstrated a state of sensitization to extrinsic apoptosis via the induction of Fas/FasL and $DR_{4/5}$/TRAIL following treatment with melatonin [131–133]. However, there are inconsistencies regarding the underlying pathways as the study of NSCLC reported a reduction of Akt activity and using glioma cell lines also demonstrated Akt inactivation in addition to PKC inhibition by melatonin while in the study on AML, ALL, and BL cell lines, increase of phosphorylated Akt levels were reported following melatonin incubations. TRAIL induction by melatonin is noteworthy regarding the attenuation of apoptosis resistance of hypoxic cancer cells and an essential part of this effect relates to the inhibition of HIF-1α, which plays a crucial role in tumor survival and apoptosis resistance under hypoxic conditions [133,134]. Rodriguez-Garcia et al. reported that melatonin induced neuroendocrine differentiation of prostate cancer cells, which resulted in an increased sensitivity to TRAIL and TNF-α induced apoptosis although the sensitivity to doxorubicin, docetaxel, and etoposide remained unchanged [135]. As melatonin has been shown to regulate the differentiation status of various cell, the contribution of this effect to cancer resistance needs to be further explored.

9.6. Hyperactive telomerase reverse transcriptase

Telomeres in cancer cells are essential for maintaining genomic stability. Erosion of telomeres, as naturally happens during cell division, destabilize the genome and predisposes it to chromosomic fusion and recognition as sites of DNA damage, which would result in senescence and apoptosis. Therefore, cancer cells with accelerated rates of division employ human telomerase reverse transcriptase (hTERT) to mend the diminution of their telomeres. In fact, hTERT which shows negligible activity in normal human cells is reactivated in over 90% of cancers [136].

As hTERT was demonstrated to be a crucial factor for tumor growth and survival, it was scrutinized regarding its possible links to tumor resistance and its implications were soon unraveled. In fact, it was revealed that telomerase expression is associated with radiotherapy resistance [137] and that decreased hTERT is correlated with sensitization of cancer cells to various chemotherapeutic agents. hTERT may confer resistance to cancer cells via a number of mechanisms. hTERT translocates to mitochondria where it exerts protective effects possibly via a reduction of mtDNA damage and ROS generation. Furthermore,

increased co-expression of hTERT and ABC transporters has been reported in melanoma cell lines [138]. The increased expression of hTERT could also suppress p53-mediated cell death, thus making cells resistant to DNA damaging agents [139] Additionally, telomerase was reported to induce resistance to various insults such as ionizing radiation, hydrogen peroxide, bleomycin, and etoposide possibly via its telomere elongating ability [140,141]. Numerous strategies have already been suggested for the inhibition of hTERT, with the aim of sensitization of cancers [142] and based on the reports of the studies reviewed so far, melatonin is also a suppressor of hTERT expression. Futagami et al. first reported that the combination of melatonin with CDDP decreases ovarian cancer cell proliferation. They also observed that this combination could also decrease telomerase activity in OVCAR-3 cell lines [143]. Later studies reported that the combination of melatonin with cisplatin on hepatocellular carcinoma and with berberine on NSCLC, enhance the inhibition of hTERT. This was due to the decreased expression and activity of AP-2β [121,124], which is a transcription factor regulating hTERT by binding to its promoter [144].

9.7. Autophagy

Autophagy is a catabolic process in which targets such as macromolecules or even organelles, such as mitochondria, undergo lysosomal degradation [145]. Autophagy plays a dual role regarding the survival of cancer, while it can promote the survival of cancer cells and increase their resistance to various insults including chemotherapy, it is also an alternate means of programmed cell death, which can aid in the elimination of malignant cells. A growing body of studies demonstrates the role of autophagy induction in resistance to chemotherapy, and as of now, there are numerous clinical trials of autophagy inhibitor combinations with chemotherapy to increase the responsiveness of cancers [146]. However, in certain conditions, the contribution of autophagy in mediating cell death in resistant cancer cells has been noted. One such example is the growth inhibition and induction of autophagic cell death in tamoxifen-resistant MCF-7 tumors by a HDAC inhibitor [147]. Rather similar observations were made In dLEN exposed MCF-7 xenografts, where the supplementation of melatonin induced autophagy, which might have contributed to tumor regression [84]. It appears that induction of mitophagy by melatonin is, in part, involved in its potentiation of cancer sensitivity. In fact, melatonin could synergistically enhance the antineoplastic effects of sorafenib in hepatocellular carcinoma via mitophagy enhancement, possibly due to increased ROS generation and mitochondrial depolarization. Addition of melatonin to sorafenib stimulated the colocalization of mitochondria and lysosomes, enhanced the expression of PINK1 and Parkin, which are the primary factors conduction of mitophagy, and reduced the amount of mt-DNA in treated cells, which display an increased degradation of mitochondria by mitophagy [148]. Overall, considering the conflicting role of autophagy in cancer besides the fact that melatonin can both enhance and diminish autophagy and mitophagy depending on the type of cells and treatment condition, it is not possible to reach a general consensus regarding the contribution of modulating autophagy in sensitization or resistance of cancers, and with respect to type of cancer and surrounding circumstances, future studies are warranted as this subject is of profound importance. A summary of the mechanisms involved in drug resistance and the role of melatonin are presented in Fig. 2.

9.8. Glycolysis

It has been quite a long time since Warburg first described in 1956 the respiratory injury of cancer cells and the increase of the so called fermentation process by these cells to compensate for the cellular energy defect; a phenomenon referred to as the Warburg effect [149]. Normally within a healthy cell, pyruvate is formed during glycolysis in the presence of adequate amounts of oxygen where it mostly enters the mitochondria to dispatch to the tricarboxylic acid (TCA) cycle (aerobic
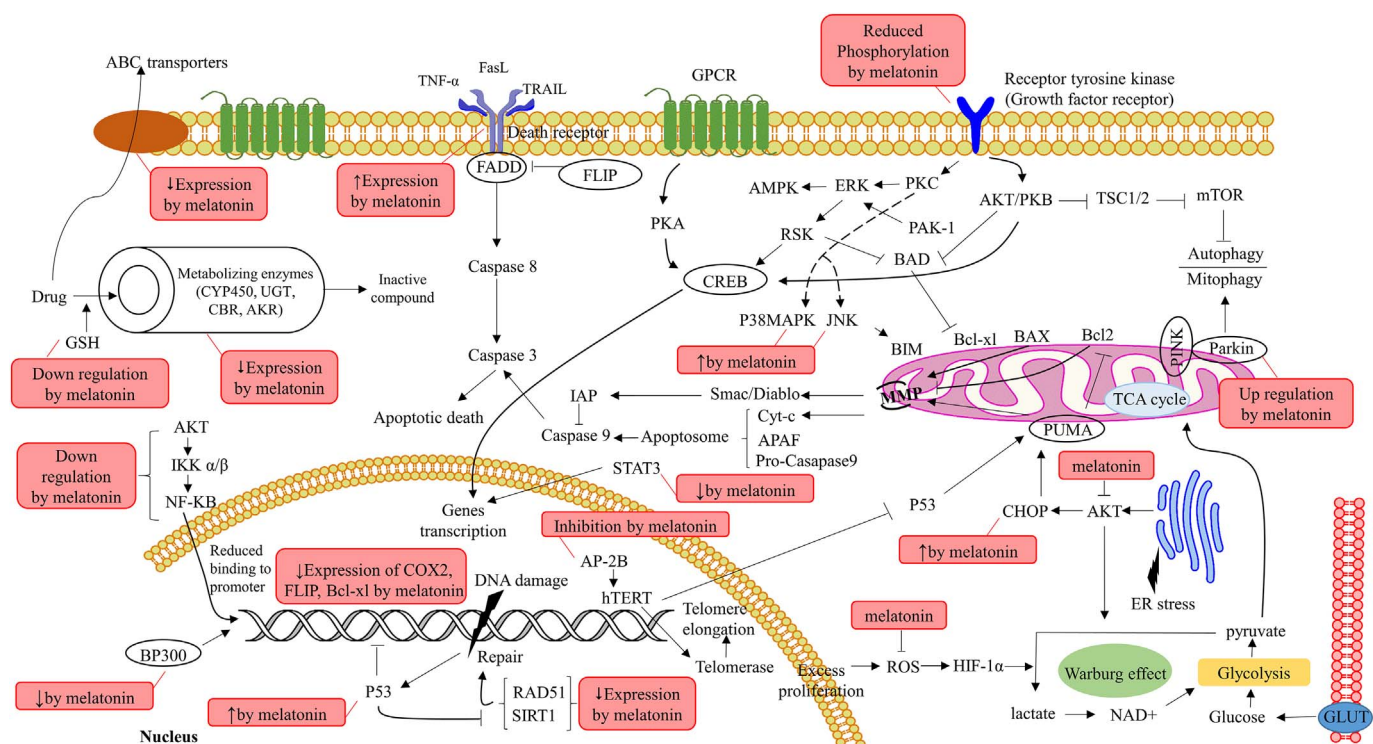
Fig. 2. Mechanisms involved in drug resistance and the role of melatonin.

glycolysis); while under conditions of oxygen insufficiency, the cells are mostly inclined to convert pyruvate to lactate (anaerobic glycolysis). Many cancer cells undergo metabolic reprograming during which the conversion of pyruvate to lactate is predominantly occurred even in the presence of oxygen to provide the required components of macromolecule synthesis during rapid cancer cell proliferation [150]. Melatonin has been shown to inhibit cancer cell proliferation via several mechanisms among which the suppression of aerobic glycolysis is addressed herein. It is been both experimentally and clinically reported that melatonin supplementation at low doses can affect glucose metabolism via a series of direct and indirect effects. First, it can repress the Warburg effect in cancer cells as suggested to be mediated by its inhibitory effect on p-AKT, a major regulator of aerobic glucose metabolism [151]. Second, we know that melatonin possesses potent antioxidant properties and that the accumulation of reactive oxygen species (ROS) can trigger HIF-1α activity. On such a basis, melatonin can alleviate the HIF-1α induced activation of the Warburg effect in the cell by scavenging the mitochondrial ROS and thus indirectly reduce the anaerobic glycolysis which is a survival tool exploited by cancer cells [152]. Since these effects of melatonin were completely reversed following treatment with the unspecific MT1/MT2 inhibitor S20928, it has been shown that melatonin exerts the aforementioned effects in a receptor-dependent manner [151].

10. Concluding remarks

Reports from the studies so far demonstrate that melatonin possesses the capability to sensitize various cancers, such as breast, lung, colon, hepatic, and hematologic cancers to the effects of antineoplastic agents like anthracyclines, alkylating agents, tyrosine kinase inhibitors, endogenous factors such as TNF-α, TRAIL, and FAS, as well as ionizing radiation. Melatonin involves a collection of mechanisms in overcoming resistance and sensitization of cancer cells; (a) modulation of target receptors of drugs, (b) reduction of cellular clearance of medications by downregulation of transport mechanisms and metabolizing enzymes (c) impairment of DNA repair (d) enhancement of

programmed cell death via apoptosis and autophagy (e) modulation of signal transduction pathways involved in promotion of survival. The pleiotropic actions of melatonin are particularly valuable since cancer cells commonly employ multiple mechanisms for their survival and resistance to therapy. The ability of melatonin to selectively sensitize cancer cells to cytotoxic therapies while protecting normal cells from toxicities of such agents, warrant its consideration as a potential adjuvant to cancer treatment and further research in this field is encouraged.

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