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Cancer metastasis: Mechanisms of inhibition by melatonin

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Abstract

Melatonin is a naturally occurring molecule secreted by the pineal gland and known as a gatekeeper of circadian clocks. Mounting evidence indicates that melatonin, employing multiple and interrelated mechanisms, exhibits a variety of oncostatic properties in a myriad of tumors during different stages of their progression. Tumor metastasis, which commonly occurs at the late stage, is responsible for the majority of cancer deaths; metastases lead to the development of secondary tumors distant from a primary site. In reference to melatonin, the vast majority of investigations have focused on tumor development and progression at the primary site. Recently, however, interest has shifted toward the role of melatonin on tumor metastases. In this review, we highlight current advances in understanding the molecular mechanisms by which melatonin counteracts tumor metastases, including experimental and clinical observations; emphasis is placed on the impact of both cancer and non-neoplastic cells within the tumor microenvironment. Due to the broad range of melatonin's actions, the mechanisms underlying its ability to interfere with metastases are numerous. These include modulation of cell–cell and cell–matrix interaction, extracellular matrix remodeling by matrix metalloproteinases, cytoskeleton reorganization, epithelial–mesenchymal transition, and angiogenesis. The evidence discussed herein will serve as a solid foundation for urging basic and clinical studies on the use of melatonin to understand and control metastatic diseases.

KEYWORDS

angiogenesis, epithelial–mesenchymal transition, matrix metalloproteinase, melatonin, metastasis

1 | INTRODUCTION: CANCER METASTASIS

Cancer metastasis, which accounts for most deaths due to malignancies, is a multistage process that requires cancer cells to escape from the primary site, survive in the circulation, and develop in distant tissues.[1] As cancer cells grow at the primary site, formation of new capillary structures (a process known as angiogenesis) provides not only oxygen and nutrients but also a route by which a subset of tumor cells with the ability to self-renew and actively migrate[2] (defined as cancer stem cells) can penetrate the basement membrane and extracellular matrix (ECM). These motile cells undergo intravasation to enter the vascular circulation. Additionally, tumor cells might indirectly enter the blood circulatory system after their entrance into lymphatic vessels. Once malignant cells survive in the detached state in the circulation, they then may exit the circulation via the process of extravasation to invade the vascular basement membrane and ECM.[3] To form a secondary tumor, a premetastatic niche needs to be established for the disseminated cells to ultimately attach and grow.[4] Each of these processes is orchestrated by the inherently complex genomic aberrations in late-stage cancers[5] and heterotypic interactions between tumor and stromal cells

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within the tumor microenvironment.[6] A better understanding of tumor metastases is crucial not only for unraveling the mechanisms underlying cancer progression but also for improving the development of cancer treatment and patient prognosis (Figure 1).

2 | MELATONIN AS AN ANTIMETASTASIS AGENT

2.1 | Melatonin: mechanisms of action

Melatonin (N-acetyl-5-methoxytryptamine), first identified as a neurohormone in the bovine pineal grand,[7] is produced in a great variety of tissue types including the brain, retina, lens, cochlea, Harderian gland, airway, skin, gastrointestinal tract, liver, kidney, thyroid, pancreas, thymus, spleen, lymphocytes, and in the reproductive tract.[8–10] In the pineal gland, its synthesis and secretion is controlled by the prevailing light:dark cycle as detected by the eyes where light represses and darkness augments its production.[11,12] Melatonin has multiple means to carry out its effects. Melatonin acts via membrane G protein-coupled receptors (MT1 and MT2) that are expressed in numerous areas of the central nervous system and in peripheral tissues to modulate diverse biological activities.[13–16] One of the best-described membrane receptor-mediated actions involve an influence on phasing of circadian rhythms and sleep promotion.[17,18] Additionally, other actions of melatonin are attributed to its ability to bind to nuclear and cytoplasmic interacting partners.[19] For example, melatonin is a natural ligand for the retinoid-related orphan nuclear hormone receptor family (RZR/ROR).[20] Melatonin's immunomodulatory effects and some of its circadian actions are mediated through these nuclear interactions.[21,22] Furthermore, melatonin interacts with calmodulin,[23,24] an intracellular protein implicated in cytoskeletal rearrangement[25] and estrogen signal pathways.[26] The cytosolic binding partner of melatonin is the enzyme quinone reductase 2; this enzyme protects against oxidative stress by preventing electron-transfer reactions of quinones.[27,28] Finally, melatonin and its metabolites are potent scavengers of both reactive oxygen (ROS) and reactive nitrogen species.[29–32]

Mitochondria are the major site of free radicals and related toxic species generation. Melatonin has been consistently shown to not lessen the formation of ROS/RNS at the mitochondrial level, thereby protecting against oxidative or nitrosative damage to electron transport chain proteins; it also limits lipid peroxidation in the inner membrane, thus favoring electron flux and ATP production.[33,34] It was also recently suggested that the mitochondria of all cells may be the site of melatonin synthesis.[35]

Various lines of evidence have suggested pleiotropic roles of melatonin in the maintenance of health.[36–38] A reduction in circulating melatonin levels, polymorphisms of melatonin receptor genes, and circadian disruption are associated with a myriad of physiological and pathological disorders including aging, metabolic syndrome, type 2 diabetes, immune diseases,

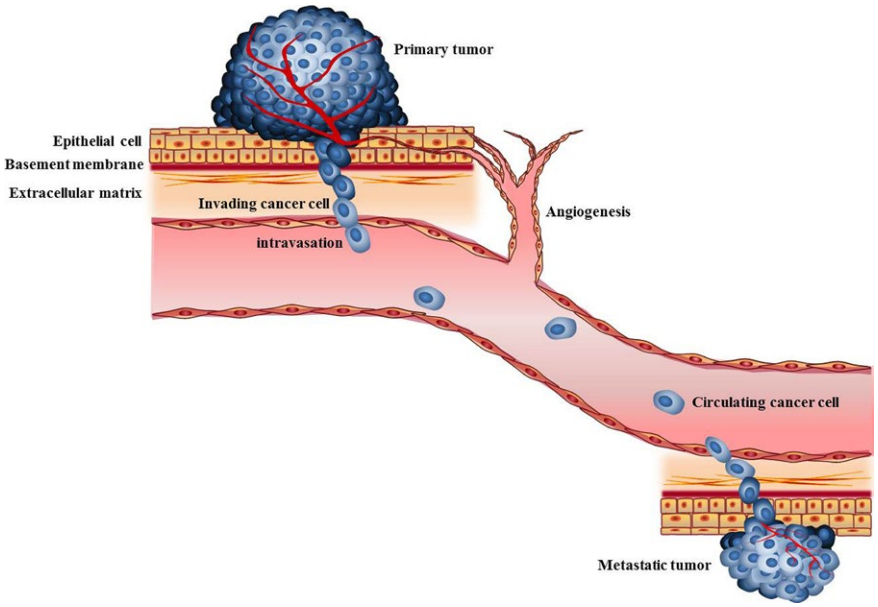

FIGURE 1 The process of tumor metastasis. Only a subset of malignant cells from a primary tumor acquires invasive and migratory properties. Cells that escape from the primary tumor invade the extracellular matrix and pass through the basement membrane of endothelial cells. Some of these cells enter the bloodstream in a process called intravasation, that is, they pass between (diapedesis) cells of the endothelial monolayer lining the interior surface of vascular wall. Using the bloodstream spread throughout the body, disseminated cells then exit the circulation in a process called extravasation at potential secondary tumor site. Many of the cancer cells that extravasate do not successfully initiate a new tumor but rather undergo cell death. The remaining cells that survive can then enter a state of dormancy or proliferate to give rise to metastases

hypertension, several mood and cognitive disorders, and cancer.[39–46] Melatonin, under both in vitro and in vivo conditions, inhibits the growth of numerous cancer types.[47–50] Due to its broad spectrum of actions, there are multiple mechanisms underlying melatonin's ability to counteract tumor growth. These include, but are not limited to its varied antioxidant effects, modulation of the cell cycle, induction of apoptosis, inhibition of telomerase activity, ability to antagonize metastasis, prevention of circadian disruption, anti-angiogenesis, epigenetic effects, inhibition of growth factor uptake, stimulation of cell differentiation, and activation of the immune system.[51,52] As a result, melatonin has emerged as an appealing therapeutic target for anticancer therapies with consistent effects on solid tumors over a wide range of doses and cancer types.[53] In this review, we discuss recent advancements in understanding the molecular mechanisms by which melatonin inhibits tumor metastases (summarized in Table 1), including experimental evidence and clinical observations, with a focus on the impact of both cancer and non-neoplastic cells within the tumor microenvironment. It is hoped that the knowledge compiled herein will serve as a solid foundation to encourage the use of melatonin to control metastatic diseases.

2.2 | Modulation of cell–cell and cell–matrix interaction

Metastasis is the development of secondary tumors distant to the primary site. A key characteristic of metastatic cells is the substantial flexibility in their adhesive interactions with adjacent cells or with ECM components. During cancer cell invasion, the breakdown of cell–cell adhesion allows tumor cells to dissociate from the primary tumor mass, and alterations in cell–matrix interaction potentiate the ability of the cells to invade the surrounding stroma.[54] The cell architecture of epithelial cells is characterized by a remarkable polarization of the plasma membrane, maintained by a variety of compositionally and functionally distinct surface structures. These cell-to-cell adhesion complexes include tight junctions (TJs), adherens junctions (AJs), gap junctions (GJs), desmosomes, and hemidesmosomes. Among these, melatonin exhibits an inhibitory role on the invasive properties of cancer through regulating the expression of cell adhesion molecules associated with TJ and AJ.

A hallmark alteration of the cell junction in the early step of metastasis involves the loss by cancer cells of E-cadherin, a prototypical member of the type-1 classical cadherins found at AJs.[55] When forming AJs with adjacent epithelial cells, E-cadherin facilitates the congregation of epithelial sheets and retains the quiescence of the cells within these sheets. Reduced expression of E-cadherin was recognized as a prerequisite of invasion and metastasis, whereas augmentation of its expression was shown to impede the development of cancer phenotypes. In addition to its ability to alter the intercellular contacts, loss of E-cadherin contributes to metastatic dissemination by the activation of multiple signaling pathways and induction of numerous transcription factors via its intracellular binding partner, β-catenin.[56,57] Melatonin shifts one human breast cancer cell line, MCF-7, to a lower invasive status by increasing E-cadherin expression.[58] Recently, the detailed mechanism by which melatonin modulates

TABLE 1 Mechanisms by which melatonin inhibits cancer metastasis

Mechanism	Action	Cancer type	References
Cell–cell and cell–matrix interaction	Upregulation of E-cadherin	Breast and gastric cancer	58,59,103
	Upregulation of occludin	Lung cancer	61
	Upregulation of β1 integrin	Breast cancer	58
	Downregulation of αvβ3 integrin	Glioma	64
ECM remodeling	Reduced expression or activity of MMP-9	Gastric, breast, renal, oral cancer and nasopharyngeal carcinoma	73,76,77, 78,138
	Reduced expression or activity of MMP-2	Breast cancer	138
Cytoskeleton rearrangement	Microfilament reorganization	Breast cancer	82
	Microtubule reorganization	Breast cancer	82
	Downregulation of vimentin	Breast cancer	103
EMT	Downregulation of Snail and Slug	Gastric cancer	59
	Attenuation of Wnt/β-catenin signaling	Gastric cancer	59
	Attenuation of HER2-Rsk2 signaling	Breast cancer	109
Angiogenesis	Downregulation of VEGF	Gastric, prostate, and brain cancer	116-119
	Downregulation of endothelin-1	Colon cancer	124
	Reduced endothelial proliferation		126
	Decreased vascular permeability		127

ECM, extracellular matrix; MMP, matrix metalloproteinase; EMT, epithelial–mesenchymal transition; HER2, human epidermal growth factor receptor 2; VEGF, vascular endothelial growth factor.

E-cadherin induction was unraveled in an investigation into gastric cancer dissemination.[59] Melatonin-mediated upregulation of E-cadherin was demonstrated to involve with interference with the interaction between C/EBPβ and NF-κB through the action of calpain induced by endoplasmic reticulum stress.

Another cell junction involved in melatonin-regulated inhibition of cancer invasion and metastasis is TJ. As the most apical structure of epithelial cell junctions, TJs are well known as a control for the paracellular diffusion of ions and various molecules, posing a crucial role in maintaining cell integrity. Alterations in the expression and/or distribution of TJ proteins can lead to perturbations in cohesion of the TJ structure, which in turn allows cancer cells to become invasive and then ultimately result in metastasis.[60] Melatonin represses the migration of a human lung adenocarcinoma cell line, A549, which is accompanied by the upregulation of occludin, a transmembrane protein found in the TJ.[61] Occludin is required for the leading-edge localization of polarity proteins, aPKC-Par3 and PATJ, and promotes directional migration of epithelial cells by regulating membrane-localized activation of PI3K.[62]

The expression of genes encoding the adhesion molecules involved in cell-to-ECM interaction is also demonstrably altered in invading cancer cells in response to melatonin. A notable example is the integrin receptors, a group of heterodimeric cell-surface glycoproteins that function in linking molecules of the extracellular space to the intracellular actin cytoskeleton.[63] These integral membrane proteins contain two subunits, termed the α- and β-subunits. There are at least eighteen α-subunits and eight β-subunits found in vertebrates, resulting in numerous combinations of different integrin heterodimers, which selectively bind to ECM proteins to activate many intracellular signal pathways and control a range of biological processes. The anti-invasive effect of melatonin on glioma cells under hypoxic conditions was accompanied by a reduced expression of $\alpha_v\beta_3$ integrin.[64] Expression of α_v integrin was shown to be correlated with and functionally involved in the maintenance of a highly migratory phenotype in prostate cancer and laryngeal and hypopharyngeal squamous cell carcinoma.[65,66] Paradoxically, in vitro invasive capacity of breast cancer cells was reportedly suppressed by melatonin via the upregulation of β_1 integrin.[58] These findings indicate that melatonin collectively contributes to a concerted cross talk between cell–cell and cell–matrix adhesion, thwarting the attempt of malignant cells to extricate themselves from the primary site and migrate into surrounding stroma.

2.3 | ECM remodeling by matrix metalloproteinases

Successful metastasis requires not only a local microenvironment to bolster the primary cancer cells to proliferate but also a premetastatic niche to allow disseminated cells to colonize and thrive at a distant location.[67] The key constituent of such niche is the ECM, a highly dynamic structure that is present in all tissues and continuously undergoes balanced remodeling. The process of ECM remodeling is mediated by specific enzymes that are responsible for ECM degradation, such as matrix metalloproteinases (MMPs). In addition to serving as a physical scaffold for adhesion proteins of cancer cells to anchor, ECM also functions as a ligand reservoir by sequestering numerous growth factors, such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF).[68] Breakdown of ECM by matrix metalloproteinases (MMPs) liberates these growth factors, which in turn promote metastatic spread by breaking down the ECM and by stimulating angiogenesis and lymphangiogenesis.[69–71]

Melatonin exhibits versatile regulatory actions on MMP gene expression and activity in addition to its anti-inflammatory and antioxidant properties.[72] An inhibitory effect of melatonin on the induction and catalytic activity of MMP-9 in a gastric adenocarcinoma cell line has been demonstrated.[73] Melatonin interferes with MMP-9 activity through directly docking into the active site of MMP-9 and interacting with key residues within the catalytic site including three histidines that form the coordination complex with the catalytic zinc as well as proline 421 and alanine 191. MMP-9 is a zinc-dependent endopeptidase for gelatin and collagen and mainly participates in the angiogenic switch necessary for tumor development.[74] Its expression levels are associated with cancer invasion and metastasis.[75] The detailed mechanisms through which melatonin represses the expression of MMP-9 have been revealed. In a study of melatonin-regulated renal cancer metastases, melatonin inhibited NF-κB-mediated MMP-9 transcription and cancer cell invasion by targeting the Akt-Erk/JNK pathways.[76] In addition, an epigenetic regulation of melatonin on MMP-9 expression has been proposed in an investigation into oral cancer and nasopharyngeal carcinoma cell migration induced by a phorbol ester, TPA.[77,78] By reducing histone acetylation on the promoter of the MMP-9 gene, melatonin attenuates MMP-9 expression to hinder the motility of oral cancer cells. These results indicate a promising role for melatonin to constrain cancer invasion and metastasis by MMP-9-dependent ECM turnover.

2.4 | Cytoskeleton reorganization

As discussed above, the dynamic interplay between cell–cell and cell–matrix adhesion contributes to the plasticity of cancer cells that allows them to respond to external cues, from either ECM components or ECM-associated molecules. These signaling events subsequently orchestrate the changes in the organization of cytoskeletal proteins, which are essential to drive directional cell migration and invasion. The cytoskeletal elements, composed of actin microfilaments, microtubules

and intermediate filaments, are extensively integrated and play a key role in controlling cell shape and movement. Actin microfilaments and microtubules are dynamic polymer structures that are capable of organizing cytoplasmic organelles and intracellular compartments, determining cell polarity and generating both expansion and contractile forces.[79] During cell migration, these subcellular organelles affect the stabilization of cell–cell and cell–matrix adhesion through their interactions with cadherins and integrins, respectively, and generate protrusive forces at the front and retraction forces at the rear.[80] Their reorganization is central to the cross talk between E-cadherin and integrins, which is regulated by the phosphorylation of two key kinases: myosin light-chain kinase (MLCK) and Rho-associated protein kinase (ROCK), a downstream effector of Rho GTPase.[81] These two kinases are pivotal in regulating the collective migration of cells by controlling the cytoskeletal rearrangement associated with the two adhesion types.

Increased expression of E-cadherin and β_1 integrin is involved in the slowing of breast cancer cell migration by melatonin.[58] A follow-up study further demonstrated that melatonin inhibits cancer cell invasion and metastasis via ROCK-regulated microfilament and microtubule organization that converge in a migration/anchorage switch in the migrating leader cells.[82] The ROCK family consists of two isoforms, ROCK1 and ROCK2, sharing 65% overall homology and 92% homology in the kinase domain. Both kinases play primary roles in the organization of the cytoskeleton and are implicated in a wide range of fundamental functions in cancer progression.[83] In addition to interfering with the kinase activity, melatonin is effective in restraining cancer migration through the downregulation of ROCK-1[84,85] and MLCK.[61] These findings highlight a role of melatonin in cytoskeleton rearrangement by targeting several key signaling molecules during cancer metastasis.

2.5 | Epithelial–mesenchymal transition

The morphogenesis process of epithelial–mesenchymal transition (EMT), in which epithelial cells shift toward the mesenchymal state and become migratory, was first recognized as a principal process in embryonic development and organogenesis (Figure 2).[86] Recently, mounting evidence has indicated that manipulation of EMT also affects cancer progression and metastasis.[87,88] The molecular and cellular changes in EMT are characterized by a loss of epithelial cell–cell junctions and apical–basal polarity, rearrangement of cytoskeleton, and gain of migratory phenotypes.[89] This switch in cell architecture and behavior is mediated by a series of transcriptional and signaling events elicited from various extracellular stimuli.[90] Signaling pathways, such as NF-κB, Wnt, Notch, Hedgehog, AP-1, and growth factor signaling, induce or modulate the EMT process.[91–93] Many of these signal cascades converge at the level of EMT-associated

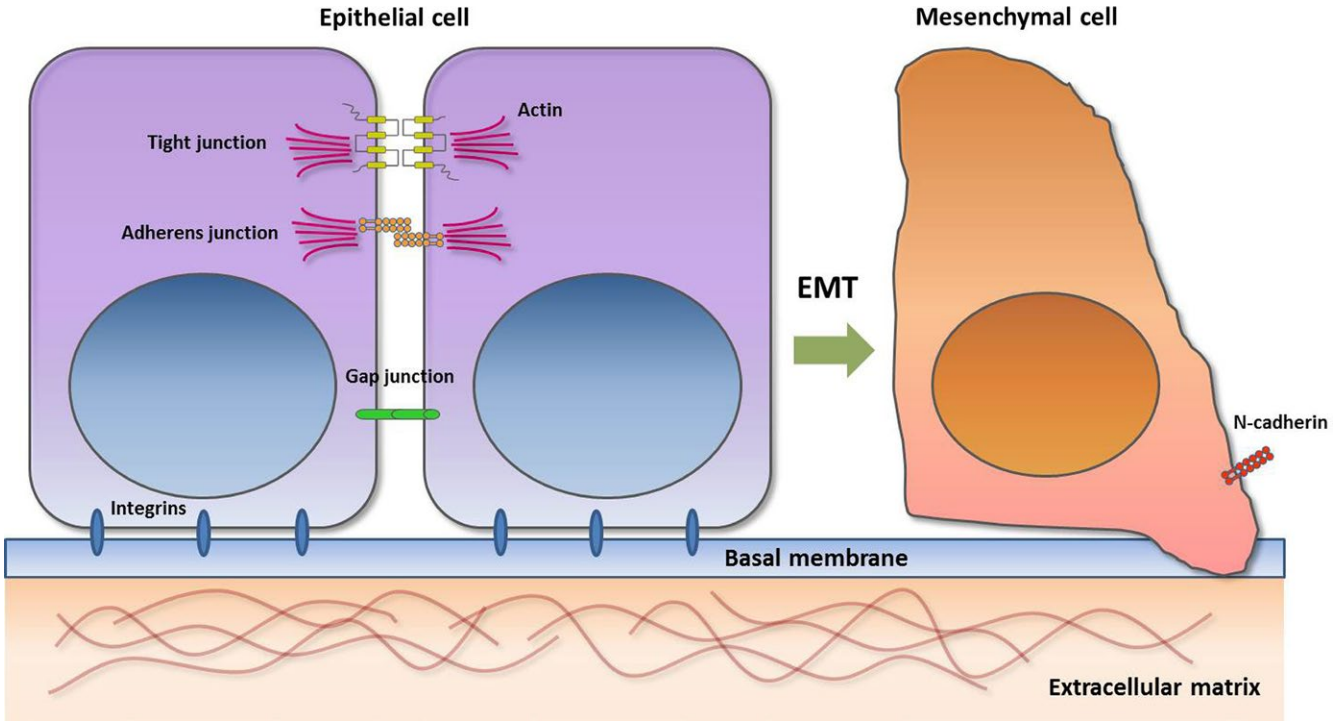

FIGURE 2 Schematic representation of the epithelial–mesenchymal transition (EMT). The morphogenesis process of EMT, in which epithelial cells shift toward the mesenchymal state and become migratory. EMT occurs when epithelial cells lose their epithelial cell characteristics, including dissolution of cell–cell junctions, that is, tight junctions and adherens junctions

transcription factors, of which Snail, Slug, Twist, and Zeb are commonly unregulated in metastatic cells that are undergoing EMT;[87] they function to regulate a hallmark of EMT and cancer metastasis, the downregulation of E-cadherin.[94–98]

Melatonin impedes the EMT process and cancer cell dissemination through interference with NF-κB signaling.[59] The effects of NF-κB on melatonin-mediated inhibition of EMT are partly attributed to its direct and indirect regulation of EMT-associated transcription factors, Snail, Slug, Twist, and Zeb,[99] which negatively control the expression of E-cadherin. In addition to suppressing an epithelial phenotype, NF-κB also contributes to the induction of vimentin,[100–102] a cytoskeletal protein vital for cell migration, and promotes and maintains a mesenchymal state. Decreased expression of vimentin was observed when the migration and invasion rate of mammospheres formed by breast cancer cells was repressed after melatonin treatment (NF-κB inhibition).[103] Also upregulated by NF-κB to display metastatic behaviors is another mesenchymal marker, MMP-9.[104–106] An inverse correlation between melatonin receptor 1A (MTNR1A also known as MT1) and MMP-9 expression in renal cancer and normal kidney tissues has been noted.[76] By targeting NF-κB and Akt-MAPK pathways, melatonin inhibited MMP-9 transactivation and renal cancer metastasis. These data collectively link NF-κB to melatonin's actions on curbing the EMT and cancer metastasis.

The Wnt/β-catenin pathway represents another key regulator of the EMT. β-catenin remains a core element of the adherens junction by virtue of its interaction with E-cadherin.[107] During the EMT, loss of E-cadherin leads to the dissociation of β-catenin from the junctional complex. As such, β-catenin then translocates to the nucleus and binds to the T-cell factor/lymphocyte enhancer factor (TCF/LEF), a main transcription factor that turns on the expression of Wnt target genes, including Snail and Slug. In the absence of Wnt signaling, a destruction complex, composed of glycogen synthase kinase 3 β (GSK3β), axin, adenomatous polyposis coli (APC), and casein kinase 1, phosphorylates excess cytoplasmic β-catenin and targets it for ubiquitin-dependent degradation. In a xenograft model of breast cancer, the phosphorylation of GSK3β, resulting in inhibition of its activity, is regulated in a circadian manner.[108] Melatonin activates GSK3β by interfering with Akt phosphorylation to induce β-catenin turnover and hinders the EMT, revealing a promising role for circadian gating of EMT in metastatic spread via regulation of GSK3β.

Recently, Rsk2, a key signaling node activated by the HER2/MAPK/Erk pathway, was identified as being involved in the melatonin-mediated suppression of EMT and late-staged metastasis in breast cancer cells.[109] HER2 is a member of the human epidermal growth factor receptor (HER) family that plays an important role in the progression of certain aggressive types of breast cancer.[110] This finding provides insight into a therapeutic use of melatonin in patients with HER2-positive breast cancer.

2.6 | Angiogenesis

An extensive vascular network is required in the tumor mass to provide the supply of oxygen and nutrients and the route for malignant cells to escape from the primary site. The process whereby new blood vessels form from pre-existing ones is called angiogenesis. Tumor angiogenesis requires complex interactions between endothelial cells and the microenvironment, allowing the endothelial sprouts to grow, convert into endothelial tubules, and connect with other vessels.[111] Several key signaling pathways have been defined that mediate angiogenesis.[112] This list includes, but is not limited to, growth factors, integrins, cell-surface receptors, cytokines, chemokines, lipids, and the ECM.

Vascular endothelial growth factor (VEGF) is a potent pro-angiogenic factor thought to be crucial for tumor angiogenesis and metastasis.[113] As the VEGF family is present not only in cancer but also in the adjacent non-neoplastic cells, such as endothelial cells, it has emerged as a therapeutic target in anticancer treatment.[114] The anti-angiogenic activity of melatonin is related to a decline in VEGF secretion in patients with advanced cancer.[115] Various studies using cancer cell lines demonstrated that melatonin destabilized the transcription factor, hypoxia-inducible factor-1α (HIF-1α), which enhances the expression of VEGF.[116–119] Several distinct mechanisms underlying melatonin's actions in reducing the levels of HIF-1α and VEGF have been proposed. In a mouse model of renal cancer, the action of melatonin on HIF-1α and VEGF expression was associated with its antioxidant activity and independent of its membrane receptors.[115] Such inhibition of HIF-1α-induced transcription is explained by the altered activity of the ubiquitin ligase, von Hippel–Lindau (VHL) protein, which recognizes HIF-1α as a substrate and remains part of the oxygen-sensing mechanism of the cell.[120] Another line of evidence, however, related melatonin-mediated inhibition of HIF-1α and VEGF to its interaction with the nuclear receptor RZR/RORγ in human gastric cancer cells.[119] In addition, a post-transcriptional regulation through which melatonin modified HIF-1α and HIF-2α concentrations via changes in microRNA species has been described in prostate cancer cells.[119]

Endothelin-1 (ET-1) is a vasoconstrictor that orchestrates different stages of tumor angiogenesis.[121] Through the binding to its cognate receptors, a family of G protein-coupled receptors, ET-1 upregulates MMP-2 and VEGF in endothelial and ovarian cancer cells, respectively.[122,123] It has been reported that melatonin reduced ET-1 expression and secretion in colon cancer cells via the inactivation of FoxO1 and NF-κB,[124] revealing an additional role of melatonin in modulating angiogenesis.

In addition to acting on the cancer cells, melatonin directly exhibits anti-angiogenic effects by suppressing the proliferation and migration of endothelial cells.[125] A wide spectrum of melatonin receptors, including MT1, MT2, RORα, and RORβ, but not RORγ, was found to be expressed in a line of human primary endothelial cells, the human umbilical vein endothelial cell (HUVEC).[126] Through these receptors, high concentrations of melatonin markedly reduced HUVEC proliferation via the Erk/Akt/NF-κB pathway. Inhibition of NF-κB by melatonin in HUVEC also protected the permeability of endothelial monolayer by attenuating MMP-9 expression and activity;[127] a marked rise in vascular permeability is a hallmark of angiogenesis. These results demonstrated that melatonin functions as an anti-angiogenic agent through the reduction in VEGF secretion by cancer cells and by directly antagonizing endothelial cell activation.

3 | POTENTIAL DIRECTIONS FOR FUTURE RESEARCH

Several new concepts and mechanistic insights into the complex process of metastasis have emerged in the past few years.[1,5,128,129] Despite this, the findings of intensive studies on the antimetastatic effects of melatonin are primarily concentrated on "how" malignant cells leave the primary site, with an emphasis on alterations in cell adhesion, invasion, and migration. The impacts of melatonin, if any, on "who" can successfully navigate the metastatic process and "where" disseminated tumor cells settle remain largely elusive.

Cancer stem cells are recognized to be directly relevant to metastases;[2] as such, cells would be potential candidates for the acquisition of highly motile activities and formation of heterogeneous cancer cell populations at unwelcoming

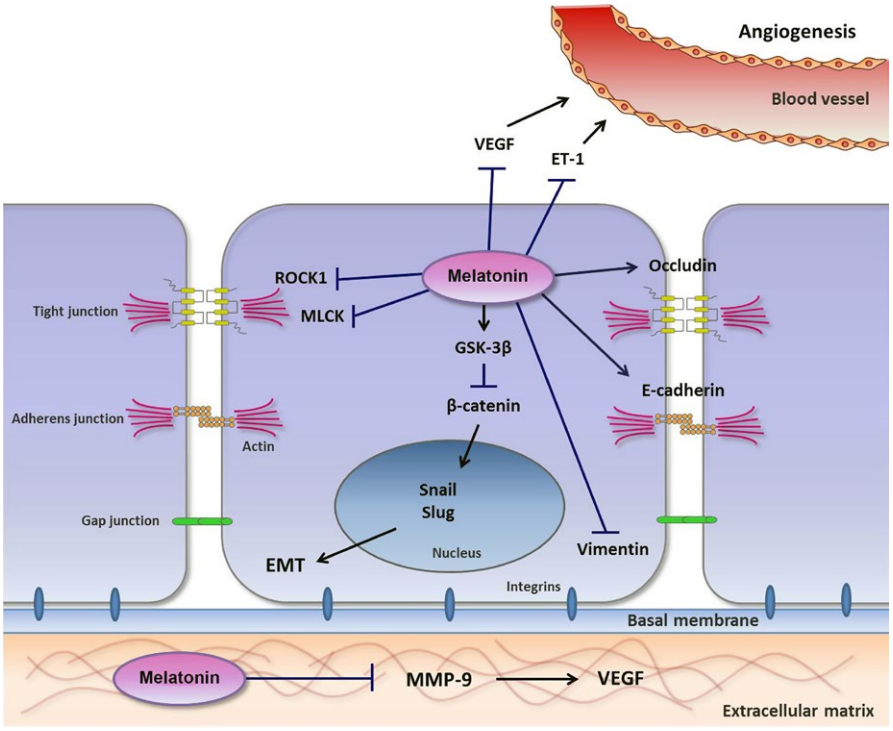

FIGURE 3 Schematic representation of the multiple mechanisms involved in melatonin-mediated inhibition of cancer metastasis. When cancer cell invasion takes place, the loss of cell–cell adhesion potentiates the ability of malignant cells to dissociate from the primary neoplasm and alterations in cell–matrix interaction allow the cells to penetrate the surrounding tissues. Melatonin shifts cancer cells to a lower invasive status by upregulation of epithelial cell adhesion molecules, such as E-cadherin, occludin, and β_1 integrin. Extracellular matrix (ECM), representing the key component of tumor microenvironment that continuously undergoes controlled remodeling by matrix metalloproteinases (MMPs), not only serves as a physical scaffold for adhesion proteins of cancer cells to anchor but also functions as a ligand reservoir of numerous growth factors, such as vascular endothelial growth factor (VEGF). Breakdown of ECM by MMPs liberates these growth factors. Melatonin attenuates the expression and activity of MMP-9 to constrain cancer invasion and metastasis. Such interplay between cell adhesion and ECM remodeling subsequently orchestrates the changes in the organization of cytoskeletal proteins because melatonin is effective in regulating cytoskeletal dynamics through the downregulation of a major intermediate filament protein of migrating cells, vimentin (VIM), and two key kinases, myosin light-chain kinase (MLCK) and Rho-associated protein kinase 1 (ROCK1). In addition, the epithelial–mesenchymal transition (EMT), characterized by a loss of epithelial cell–cell junctions and apical–basal polarity, rearrangement of cytoskeleton, and gain of migratory phenotypes, is mediated by a series of transcriptional and signaling events. Melatonin induces β-catenin turnover by activating GSK3β to limit the expression of EMT-associated transcription factors, such as Snail and Slug. Moreover, as cancer cells grow in the primary site, formation of new capillary structures (angiogenesis) is required to supply oxygen and nutrients as well as the path by which cancer cells can actively migrate. Melatonin also attenuates the expression and secretion of pro-angiogenic factors, such as VEGF and endothelin-1 (ET-1) by cancer cells to counteract angiogenesis. These mechanisms are interrelated and explain the antimetastatic properties of melatonin

tissues. Although the precise nature of cancer stem cells is not fully understood, their phenotypic properties fit the long-standing theory of metastasis, that is, the late metastasis model, where transformed cell dissemination occurs late in cancer progression and only a subset of stem-like cells can successfully migrate to colonize new sites.[130] However, this model has been challenged by the observation that patients with cancer sometimes develop metastases more than 10 years after the surgical removal of the primary tumors.[131] Thus, an alternative hypothesis, the early metastasis model, was proposed as malignant cells with a fairly normal phenotype may break away from the primary neoplasm early during tumorigenesis and stay dormant in a receptive ectopic site.[132] These hidden cells, forming clinically undetectable micrometastases, may provide a source of future cancer cells for seeding other locations and growing into macrometastases. The factors that liberate these cancer cells from dormancy are now the subject of extensive studies.[128,133] These two types of metastasis-initiating cells exhibit substantial differences in genetic similarities with the primary tumor and in response to anticancer drugs, especially for those targeting rapidly dividing cells. Recently, a study using cultured mammospheres, the growth of stem-like breast cancer cells in suspension conditions, demonstrated that melatonin treatment is effective against proliferation of human breast cancer stem cells in vitro and impacts the estrogen receptor pathway.[134] Further exploration of the potential effects of melatonin on the behavior of these two cell populations may reveal novel insights into the antimetastatic roles of this multifaceted hormone.

Another puzzle relates to the potential role of melatonin in determining the target destination of the disseminated cancer cells. It is evident that the metastatic site tropism is influenced by the establishment of the premetastatic niche, where VEGF receptor 1-positive hematopoietic progenitor cells from the bone marrow are recruited to specific sites by the cytokines and growth factors secreted by the primary tumor and form receptive clusters for the metastatic cells to arrive.[4] Such discovery prompts the notion that a conducive microenvironment at metastatic locations is important for migrating tumor cells to locate. Within this microenvironment, transactivation of MMP-9 by resident and bone marrow-derived cells regulates ECM remodeling and subsequently results in the stabilization of endothelial progenitor cells, thus promoting angiogenesis and metastatic progression.[1] As such, a role of melatonin in orchestrating communications between tumor cells, ECM, soluble mediators, and resident and recruited host cells at the metastatic, rather than the primary foci is an appealing topic for future investigations.

4 | CONCLUDING REMARKS

Melatonin treatment has been utilized in various clinical settings and has a very low toxicity profile over a wide range of doses in most cases.[135] In clinical oncology, melatonin, used as a complementary treatment, is shown to enhance the efficacy and attenuate the side effects of chemoradiotherapies.[52,136–138] It has also been found that melatonin makes cancer cells previously insensitive to chemotherapies sensitive to the therapeutic agents.[139–141]

Herein, we illustrate that melatonin suppresses tumor metastases via its regulation on cell adhesion, ECM remodeling, cytoskeleton reorganization, the EMT, and angiogenesis at the molecular, cellular, and organismic levels (Figure 3). Also discussed are potential antimetastatic actions of melatonin that remain to be elucidated. These data provide clues for further clarification of additional mechanisms underlying melatonin-mediated inhibition of metastasis and should aid in the design of clinical trials for combination therapies to combat metastatic diseases.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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