褪黑素受体及其介导的抗肿瘤机制研究进展

王雪竹 孙 铮*

(大连医科大学中西医结合基础研究所, 大连 116044)

摘要 褪黑素(melatonin, MT)是由松果腺分泌的一类神经内分泌激素, 具有维持生理节奏、增强免疫调节、抗氧化、保护唾液腺、增强肌肉力量等生理功能。有研究发现, 褪黑素可通过与其受体结合发挥抗肿瘤作用, 关于这方面的研究已成为当今热点领域。该文就褪黑素抗肿瘤机制及其受体作用的研究进展作一阐述, 通过总结有关褪黑素研究现状并进行展望, 为褪黑素临床合理、精准用药提供理论基础。

关键词 褪黑素; 褪黑素受体; 机制; 抗肿瘤作用

Advance in the Anti-Tumor Mechanism Mediated by Melatonin Receptor

Wang Xuezhu, Sun Zheng*

(*Institute of Basic Research of Integrative Medicine, Dalian Medical University, Dalian 116044, China*)

Abstract As a type of neuroendocrine hormones secreted by pineal gland, melatonin (MT) can maintain circadian rhythm, enhance immune regulation, resist oxidation, protect salivary glands, and strengthen muscle strength. Studies have found that MT can exert the effect of antitumor by combining with its receptor, and the research on this subject has become the current hotspot. In this review, the research progress of the receptor function of MT and its antitumor mechanism are discussed. Through summarizing the research status and future prospect of MT, some theoretical basis is provided for the clinical reasonable and accurate medication of MT.

Keywords melatonin; melatonin receptor; mechanism; anti-tumor effect

1959年, 国外学者从牛的松果体中分离得到一种激素, 将其添加到青蛙的饲喂食料中可将蛙皮肤背部深色褪去, 因此将这种激素命名为褪黑素(melatonin, MT)。褪黑素是一种吲哚类激素, 化学成分为N-乙酰-5-甲氧基色胺, 在动物和人体内均广泛存在。该化合物的合成部位主要为松果体, 除此之外, 在胆汁、骨髓、胃肠道及视网膜等部位均可合成。褪黑素在机体中半衰期短, 毒性低且无法蓄积, 生理作用范围广, 具有抗氧化、增强免疫调节、调节生物钟、增强肌肉力量、抗癌等功效[1-2]。已有研究发现, 褪黑素主要通过以下方式发挥生理作用: (1)与褪黑素膜受体结合, 抑制腺苷酸环化酶(adenylyl cyclase, AC)活性, 降低环磷酸腺苷(cyclic adenosine monophosphate, cAMP)水平, 对抗雌激素以发挥抗瘤效应[3-5]; (2)与褪黑素核受体结合, 发挥免疫调节作用并参与细胞生长和骨的分化[6]; (3)对抗钙调蛋白, 引起肿瘤DNA断裂, 发挥抗肿瘤作用[7]; (4)抗氧化特性, 预防由化学性和放射性物质引发的致癌作用[8]。由于褪黑素含有亲油基团, 能够迅速通过细胞膜、细胞核膜, 并且多种组织细胞的细胞膜中都存在褪黑素高亲和力受体[9], 为其直接发挥作用提供了生理基础。Tamarkin等[10]首次观察到褪黑素能够显著抑制乳腺癌细胞增殖, 这一发现开创了褪黑素抗肿瘤研究的先河。现代生物医学研究表明, 褪黑素作用受体及相关信号转导通路与癌细胞的增殖、分化、凋亡有着密切联系。近年来, 褪黑素及其受

收稿日期: 2016-09-29 接受日期: 2016-12-15
中国博士后科学基金面上项目(批准号: 2013M541232)资助的课题
*通讯作者。Tel: 0411-86110192, E-mail: sunclank@163.com
Received: September 29, 2016 Accepted: December 15, 2016
This work was supported by the Grants of China Postdoctoral Science Foundation (Grant No.2013M541232)
*Corresponding author. Tel: +86-411-86110192, E-mail: sunclank@163.com
网络出版时间: 2017-03-28 14:04:20
URL: http://kns.cnki.net/kcms/detail/31.2035.Q.20170328.1404.004.html

体与肿瘤关系的研究日益受到关注, 本文就褪黑素及其受体参与肿瘤发生发展的最新进展进行综述, 以期为褪黑素辅助临床化疗用药提供参考。

1 褪黑素概述

褪黑素的分子式为$C_{13}H_{16}N_2O_2$, 分子量是232.27, 具有两个功能基团, 为高亲脂性兼部分亲水性的化合物。褪黑素由眼球–视交叉上核(suprachiasmatic nucleus, SCN)-颈上神经节(superior cervical ganglion, SCG)-松果体途径在黑暗/光线循环中分泌(图1)。

褪黑素广泛地分布于人体中枢神经系统和外周组织, 具有在体内吸收极不稳定, 首过效应明显等特点。如肝细胞中, 褪黑素可在微粒体羟化酶(hydroxylase)的作用下, 生成6-羟褪黑素; 褪黑素还可通过血脑屏障, 在脑内代谢发生2位氧化作用, 将其吡咯环打开形成N-乙酰-5-甲氧-犬尿酸[12]。褪黑素调节细胞生物学功能时, 先与褪黑素受体结合, 激活百日咳毒素(pertussis toxin, PTX)敏感的抑制性G蛋白信号通路, 抑制cAMP形成或阻断Forskolin(cAMP激活剂)刺激引起的cAMP升高。另外, 褪黑素亦可增加某些组织中cGMP含量, 如兔的主动脉、仓鼠的视网膜、小鼠的乳腺和人的淋巴细胞等, 发挥镇静催眠、免疫调节等作用[13]。人们对褪黑素与癌细胞关系的研究始于上世纪60年代。随后, 更多的研究结果证实了褪黑素分泌水平的增加会降低人们罹患癌症的概率[14-15]。动物实验和临床研究表明, 褪黑素能够强化化疗疗效, 加速癌细胞的死亡, 延长癌症患者生存时间, 尤其针对乳腺癌、前列腺癌、结肠癌等效果更为明显[16]。并且, 褪黑素还对顺铂(diamminedichloroplatinum, DDP)抗肿瘤活性起到明显的增效作用, 究其机制可能与下调Bcl-2(B-cell lymphoma-2)表达、上调Bax(Bcl-2 associated x protein)表达有关[17]。

2 褪黑素受体

目前普遍认可的褪黑素受体分为膜受体和核受体[18]。褪黑素膜受体属于G蛋白偶联受体(G protein-coupled receptors, GPCRs)超级家族中的一员, 即对百日咳毒素敏感的G蛋白[19-20]。该类受体主要分为两种亚型: 高亲和力MT1受体和低亲和力MT2受体[7,21-23]。MT1和MT2的DNA序列具有55%的相似性, 跨膜区结构具有70%的相似性[21]。除膜受体外, 研究人员还发现, 褪黑素存在核受体RZR/ROR(retinoid Z receptor/retinoid acid receptor-related orphan receptor), 包括RORα、RORβ和RORγ三种亚型[24]。其中, RORα普遍存在于小脑、皮肤、睾

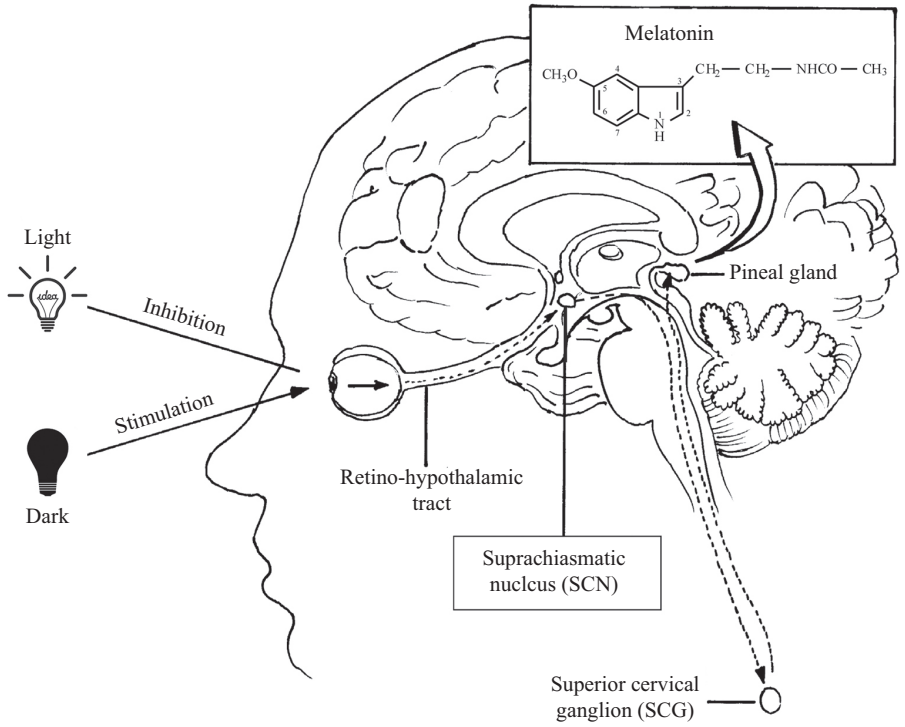


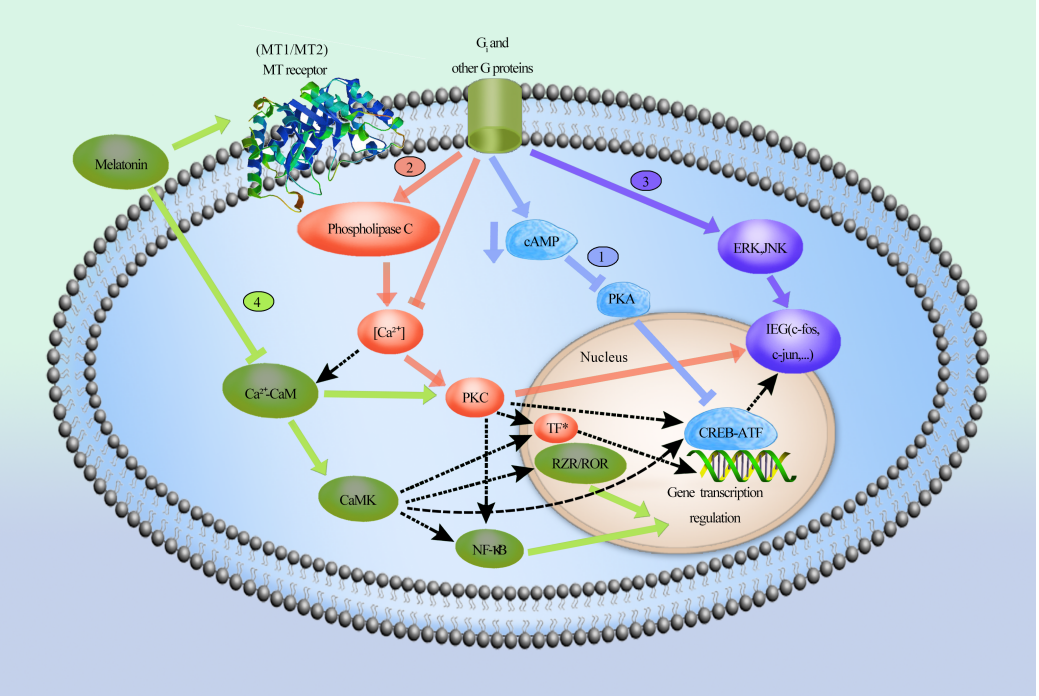
图1 肾上腺能神经支配的松果体刺激褪黑素的生成和释放(根据参考文献[11]修改)

Fig.1 Generation and release of MT stimulated by pineal gland under adrenal innervation (modified from reference [11])

丸以及人外周血白血病T细胞中[25]; 此外, 在免疫系统中, RORα一般是在淋巴系和髓系中表达。RORβ特定存在于大脑和视网膜, 而RORγ则优先表达在人体骨骼肌和胃癌细胞[26]。RORα参与抗氧化酶的表达并上调抑制炎症反应的重要标志物[如IL-6(interleukin-6)、IL-8、TNF-α(tumor necrosis factor-α)、COX-2(cyclooxygenase-2)]的水平[27]。5-脂氧酶(5-lipoxygenase, 5-LO)是一种参与肝脏炎症与纤维化的炎性酶, 褪黑素通过抑制RORα中的编码5-LO的靶基因(arachidonate 5-lipoxygenase, *ALOX5*)的表达, 调控肝星状细胞(hepatic stellate cell, HSC)的活化。以上发现为肝纤维化治疗提供了新的药物作用靶点[28]。RORβ、RORγ的功能目前尚未阐明, 有待进一步的研究。

3 褪黑素与褪黑素受体之间的相互作用

继Ebisawa等[13]于1994年首次在人体组织内发现褪黑素MT1和MT2受体表达之后, 褪黑素及相关受体发挥药理活性的机制越来越明了(图2)[29]。褪黑素与MT1结合后促使MT1的α位亚基脱落, 降低AC活性及cAMP水平, 导致ATP/ADP的合成减少, 抑制肿瘤细胞的有丝分裂, 从而发挥其抗癌作用[30]。Hill等[31]研究发现, 乳腺癌细胞中MT1受体表达水平越高, 肿瘤细胞对褪黑素敏感性越强, 这可能是由于癌细胞诱导致使MT1受体过表达, 抵抗雌激素作用, 以发挥抗肿瘤作用。与MT1受体生理功能不同, MT2



(1)褪黑素表面受体与Gi蛋白的偶联引起cAMP水平下降, 依赖性或非依赖性PKA的水平随之减少, PKA和环磷酸腺苷效应元件结合蛋白(cAMP-response element binding protein, CREB)共同调节即刻早期基因(immediate-early genes, *IEG*), 从而调节靶基因转录。(2)褪黑素结合MT1/2受体可激活磷脂酶C途径, 引起Ca^{2+}浓度升高, 可活化蛋白激酶C(protein kinase C, PKC); PKC通过两条途径: 一是活化CREB-ATF(activating transcription factor)诱导IEG表达增加, 二是活化NF-κB和其他转录因子(transcription factor, TF), 从而调节靶基因转录。(3) MT1/2受体被结合后通过G蛋白途径可直接活化细胞外调节蛋白激酶(extracellular regulated protein kinases, ERK)和c-Jun氨基末端激酶(c-Jun N-terminal kinase, JNK), ERK和JNK由胞质转移到细胞核内, 使得ATF、NF-κB(nuclear factor-κB)、c-fos、c-jun的转录增加, 从而增加蛋白质水平。(4)褪黑素抑制Ca^{2+}-CaM复合物的浓度, 抑制PKC及下游分子NF-κB、ROR(核受体)和其他TF的转录和表达。

(1) Melatonin receptors binding with Gi protein inhibits adenylate cyclase and reduces expression of cAMP, PKA and cAMP. These responses modulate *IEG* (immediate-early genes) and downstream target gene transcription, which shows the anti-cancer functions. (2) Melatonin binding with MT1/2 receptors activates the phospholipase C pathway and then up-regulates the concentration of Ca^{2+}. PKC, which is also activated, modulates the target gene transcription though two ways. Bioactive CREB-ATF induces expression of IEG, NF-κB and other transcription factors are activated. (3) MT1/2 receptors with Gi pathway activate protein kinases (ERK and JNK) directly. ERK and JNK transfer from cytoplasm to nucleus. It then makes an increase in the transcription of ATF, NF-κB, c-fos and c-jun and up-regulates the expression of the protein. (4) Melatonin inhibits the concentration of Ca^{2+}-CaM complex. This may down-regulated PKC, NF-κB(nuclear factor-κB), ROR and other transcription factor transcription and expression though CaMK. Ca^{2+}-CaM complex may also regulate PKC pathway.

图2 褪黑素作用的受体机制(根据参考文献[29]修改)

Fig.2 Receptor mechanism of MT (modified from reference [29])

受体主要发挥调节昼夜节律、血管舒张、机体免疫等作用[9]。Blask等[23]通过实验发现, 褪黑素也能对MT2受体进行正负反馈, 从而有益于胃溃疡的好转, 这种调节作用还能抑制肿瘤细胞的增殖, 进而使得肿瘤细胞凋亡。褪黑素还可参与MT2受体介导的RZR/RORα作用途径, 抑制结肠癌的增殖[32]。

除此之外, 褪黑素通过其核受体可以抑制*5-LO* mRNA的表达, 影响下游基因转录调节[28]。褪黑素仅在表达RZR/ROR同分异构体的细胞内发挥其抑制内源性5-*LO* mRNA的作用。有趣的是, 含有内源性5-*LO*表达的细胞(如B淋巴细胞和角质细胞)具有相当高的RZR/ROR的表达, 褪黑素对5-LO的下调作用还揭示了该药物可能作为基因调节剂在炎症和免疫反应中发挥重要作用。褪黑素一般通过参与*RORα*基因的表达调节炎症反应、氧化应激、癌细胞死亡和神经退化的功能[33-36]。抗炎反应中, 褪黑素与RORα结合, 通过抑制*NF-κB*(nuclear factor-κB)基因启动子的活性减少炎性细胞因子进而抑制核转录。RORα还可增加褪黑素的抗肿瘤效应, 低氧诱导因子-1(hypoxia-inducible factor-1, HIF-1)是在肿瘤进展过程中至关重要的缺氧依赖性血管生成的因素, 其表达与实体肿瘤患者的预后不良有关[26]。实验发现, 褪黑素在肿瘤缺氧微环境可视为HIF-1的抑制剂。HCT116细胞(人结肠癌细胞)中存在MT1受体与RORα的表达, 褪黑素可以时间依赖性方式下调MT1受体及上调RORα, 这可能是褪黑素抑制HCT116细胞诱导分化的原因之一[33]。综上, 深入研究褪黑素的膜受体与核受体之间的相互关系及机制对于全面了解褪黑素对细胞活动影响的分子机制存在重要价值。

4 褪黑素在恶性肿瘤发生发展中的作用及相关机制

Cohen等[33]首先提出松果体功能减退即褪黑素分泌水平下降, 可引起雌激素分泌过多, 诱发乳腺癌。随后, Tamarkin等[34]阐述了血清中褪黑素浓度与乳腺癌发生的关系。科学家还发现, 褪黑素受体(主要是MT1和MT2)也参与了肿瘤的增殖作用。我们将褪黑素发挥抗肿瘤作用归纳了以下几个方面。

4.1 抗雌激素作用

褪黑素作为肿瘤的生理性抑制剂, 尤其对激素依赖性的乳腺癌的预防以及治疗发挥重要作用[35], 这种抗乳腺癌效应最初在体外化学诱导乳腺癌大鼠模型中发现[37]。其机制为褪黑素调节雌激素水平, 首先雌激素激活腺苷酸环化酶(adenylyl cyclase, AC), 提高雌激素受体(estrogen receptor, ER)阳性细胞中的cAMP浓度, 具体表现为当褪黑素和膜受体结合后, AC活性和cAMP水平降低, 即与雌激素相互拮抗。然后, 一些与有丝分裂相关蛋白、抗凋亡蛋白Bcl-2的诱导表达下调, 同时还包括生长抑制、凋亡相关蛋白TGF-α(transforming growth factor-α)和Bax的表达[38]。褪黑素还可以抑制人乳腺癌细胞中RORα的转录活化。当RORα的表达水平提高时可协助ER招募更多的β-连蛋白(ER与RORα之间的桥梁蛋白)以及其他未知的共激活因子, 这将提高ER转录活性[39], 而RORα表达水平降低结果则相反。除了上述作用, 褪黑素还能够通过对蛋白质、原癌基因以及生长因子的调节来发挥作用, 机制总结包括: (1)抑制原癌基因*c-fos*的表达; (2)促进转化生长因子TGF-β、反作用于ER; (3)防止雌激素–雌激素受体复合物与DNA上雌激素反应元件的相互结合[40]; (4)抑制细胞增殖和细胞浸润[41]。

4.2 对钙调蛋白的作用

Sotovega等[42]发现, 褪黑素之所以能够实现其生理方面的功能, 与钙调蛋白(calmodulin, CaM)有着不可分割的联系。褪黑素对CaM发挥拮抗作用, 与磷酸二酯酶和结构蛋白(如微管蛋白)直接作用, 也可通过蛋白激酶间接影响Ca^{2+}信号转导系统, 抑制细胞增殖[43]。神经母细胞瘤中, 褪黑素通过与CaM结合选择性激活细胞中的Ca^{2+}依赖的PKCα的活性, 阻断CaM微管蛋白复合物的产生, 导致微管蛋白体积增大。钙和CaM参与癌细胞增殖中DNA的稳定性, 由此推测, 褪黑素可经此途径引起肿瘤细胞DNA断裂, 以此发挥抗肿瘤作用。

4.3 免疫调节

现今医学界普遍持有一个观点, 即维持褪黑素的正常节律性和分泌水平可延年益寿, 这可能与褪黑素增强抗体免疫功能有关[44]。褪黑素在体内促进T细胞分泌褪黑素诱导性阿片肽发挥免疫调节作用, 对抗固醇类激素或应激引起免疫抑制[1]。褪黑素的免疫作用包括体液免疫和细胞免疫两种, 可通过以下途径发挥作用: (1)褪黑素促进机体产生抗体, 提高细胞因子数量及活性; (2)增加巨噬细胞中IL-2、IL-12的表达, 通过核受体刺激淋巴细胞分泌细胞因子IL-2、IL-6; (3)褪黑素及其氧化产物可抑制脂多

糖介导的中性粒细胞中的TGF-α和IL-8的表达; (4)增加淋巴细胞的数量, 增强NK细胞毒性作用[45]。除此之外, 褪黑素还可以通过下丘脑–垂体–性腺轴、肾上腺等神经内分泌系统来发挥免疫调节作用。

4.4 对细胞亚油酸代谢的影响

Blask等[46]证实, 褪黑素在体内抑制肿瘤的主要功能成分为亚油酸(linoleic acid, LA)和ω-6多不饱和脂肪酸, LA中的ω-6多不饱和脂肪酸在乳腺癌中作用尤为明显。其中, LA的致癌作用主要表现在促进ERα的表达、调控细胞周期进展、G蛋白信号转导通路、ERK1/2途径及PI3K/Akt(protein kinase B, PKB)/mTOR(the mammalian target of rapamycin)的生长级联等[38]。研究褪黑素在不同肿瘤疾病中的作用机制时发现, 褪黑素与MT1受体结合后, MT1受体上的α亚基脱离, 抑制AC的活性, 降低肿瘤细胞的cAMP水平, 抑制LA的吸收及13羟基十八碳二烯酸[13(S)-hydroxyoctadecadienoic acid, 13-HODE]的生成。13-HODE影响某些癌细胞的有丝分裂, 当13-HODE减少时, 促有丝分裂的表皮生长因子途径(EGFR、MEK和ERK1/2途径)的磷酸化活性减弱, DNA合成和增殖被抑制。总之, 褪黑素下调LA的吸收和代谢, 进而抑制EGFR、MEK和ERK1/2途径的活化, 最终抑制肿瘤的增殖与存活[47]。此外, Akt途径也参与其中, 其与肿瘤细胞的增殖和凋亡也密切相关[17]。

5 展望

褪黑色素最大特点是来源广泛, 在植物中有大量合成, 并且对机体没有明显的毒副作用且具有脂溶性, 能够穿透血脑屏障, 提示它在治疗罹难癌症尤其是儿童期及颅内恶性肿瘤方面具有独特的药用价值。在神经肿瘤领域, 褪黑素的作用特点和分子机制也已见报道, 但其在细胞内的活性形式仍未确定。一系列体内外实验研究发现, 褪黑素可以通过CYP450酶迅速代谢成6-羟基褪黑素(6-O-Mel)、N-乙酰基-5-羟色胺(NAS), 随后, 6-O-Mel和NAS以硫酸盐和葡萄糖醛酸结合物形式经尿液排出体外。结合本课题组前期研究成果, 神经母细胞瘤和胶质母细胞瘤表现出褪黑素敏感性差异(未发表数据), 那么导致细胞敏感性差异的机制是什么、褪黑素在细胞中的活性代谢产物是什么、褪黑素代谢酶是否是影响细胞敏感差异的关键因素以及褪黑素受体是否参与其中? 这方面的相关研究仍处于空白, 而阐明这些问题是实现褪黑素的选择性用药和肿瘤个性化治疗的关键所在。有关褪黑素抗癌效果的研究日益增多, 反映出人们对该化合物药用前景的期待。目前, 褪黑素的应用已拓展到中医药学、保健学和种植等多种领域中[48]。科学家研究发现, 褪黑素受体在许多肿瘤组织中均有表达且新的作用机制仍在不断发现之中。此外, 褪黑素与其他化疗药物合用, 在治疗过程中可发挥增效、降毒等作用。本综述为褪黑素药物靶标研发、褪黑素的临床有效应用及新抗肿瘤药物的开发埋下伏笔, 对褪黑素作用机制的全面了解将有助于临床更多患者受益。

参考文献 (References)

1 Esteban-Zubero E, Garcia-Gil FA, Lopez-Pingarron L, Alatorre-Jimenez MA, Inigo-Gil P, Tan DX, *et al*. Potential benefits of melatonin in organ transplantation: A review. J Endocrinol 2016; 229(3): R129-46.

2 Hardeland R, Cardinali DP, Srinivasan V, Spence DW, Brown GM, Pandi-Perumal SR. Melatonin—a pleiotropic, orchestrating regulator molecule. Prog Neurobiol 2011; 93(3): 350-84.

3 Liu J, Clough SJ, Hutchinson AJ, Adamah-Biassi EB, Popovska-Gorevski M, Dubocovich ML. MT1 and MT2 melatonin receptors: A therapeutic perspective. Annu Rev Pharmacol Toxicol 2016; 56: 361-83.

4 Xiang S, Mao L, Yuan L, Duplessis T, Jones F, Hoyle GW, *et al*. Impaired mouse mammary gland growth and development is mediated by melatonin and its MT1G protein-coupled receptor via repression of ERalpha, Akt1, and Stat5. J Pineal Res 2012; 53(3): 307-18.

5 Mao L, Dauchy RT, Blask DE, Dauchy EM, Slakey LM, Brimer S, *et al*. Melatonin suppression of aerobic glycolysis (Warburg effect), survival signalling and metastasis in human leiomyosarcoma. J Pineal Res 2016; 60(2): 167-77.

6 Garcia JA, Volt H, Venegas C, Doerrier C, Escames G, Lopez LC, *et al*. Disruption of the NF-kappaB/NLRP3 connection by melatonin requires retinoid-related orphan receptor-alpha and blocks the septic response in mice. FASEB J 2015; 29(9): 3863-75.

7 Slominski RM, Reiter RJ, Schlabritz-Loutsevitch N, Ostrom RS, Slominski AT. Melatonin membrane receptors in peripheral tissues: Distribution and functions. Mol Cell Endocrinol 2012; 351(2): 152-66.

8 Tan DX, Hardeland R, Back K, Manchester LC, Alatorre-Jimenez MA, Reiter RJ. On the significance of an alternate pathway of melatonin synthesis via 5-methoxytryptamine: Comparisons across species. J Pineal Res 2016; 61(1): 27-40.

9 Liu J, Clough SJ, Hutchinson AJ, Adamah-Biassi EB, Popovska-Gorevski M, Dubocovich ML. MT1 and MT2 melatonin receptors: A therapeutic perspective. Annu Rev Pharmacol Toxicol 2016; 56: 361-83.

10 Tamarkin L, Cohen M, Roselle D, Reichert C, Lippman M, Chabner B. Melatonin inhibition and pinealectomy enhancement

of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in the rat. Cancer Res 1981; 41(1): 4432-6.
11 Brzezinski A. Melatonin in humans. N Engl J Med 1997; 336(3): 186-95.
12 Konturek SJ, Konturek PC, Brzozowski T, Bubenik GA. Role of melatonin in upper gastrointestinal tract. J Physiol Pharmacol 2007; 58 Suppl 6: 23-52.
13 Ebisawa T, Karne S, Lerner MR, Reppert SM. Expression cloning of a high-affinity melatonin receptor from *Xenopus* dermal melanophores. Proc Natl Acad Sci USA 1994; 91(13): 6133-7.
14 Borin TF, Arbab AS, Gelaleti GB, Ferreira LC, Moschetta MG, Jardim-Perassi BV, *et al*. Melatonin decreases breast cancer metastasis by modulating Rho-associated kinase protein-1 expression. J Pineal Res 2016; 60(1): 3-15.
15 Bizzarri M, Proietti S, Cucina A, Reiter RJ. Molecular mechanisms of the pro-apoptotic actions of melatonin in cancer: A review. Expert Opin Ther Targets 2013; 17(12): 1483-96.
16 Rozen TD. How effective is melatonin as a preventive treatment for hemicrania continua? A clinic-based study. Headache 2015; 55(3): 430-6.
17 Li W, Fan M, Chen Y, Zhao Q, Song C, Yan Y, *et al*. Melatonin induces cell apoptosis in AGS cells through the activation of JNK and P38 MAPK and the suppression of nuclear factor-kappa B: A novel therapeutic implication for gastric cancer. Cell Physiol Biochem 2015; 37(6): 2323-38.
18 Jockers R, Delagrange P, Dubocovich ML, Markus RP, Renault N, Tosini G, *et al*. Update on melatonin receptors: IUPHAR review 20. Br J Pharmacol 2016; 173(18): 2702-25.
19 Saxena VK, Jha BK, Meena AS, Narula HK, Kumar D, Naqvi SM. Assessment of genetic variability in the coding sequence of melatonin receptor gene (MTNR1A) in tropical arid sheep breeds of India. Reprod Domest Anim 2015; 50(3): 517-21.
20 Li DY, Smith DG, Hardeland R, Yang MY, Xu HL, Zhang L, *et al*. Melatonin receptor genes in vertebrates. Int J Mol Sci 2013; 14(6): 11208-23.
21 Jockers R, Maurice P, Boutin JA, Delagrange P. Melatonin receptors, heterodimerization, signal transduction and binding sites: What's new? Br J Pharmacol 2008; 154(6): 1182-95.
22 Carpentieri A, Diaz de Barboza G, Areco V, Peralta Lopez M, Tolosa de Talamoni N. New perspectives in melatonin uses. Pharmacol Res 2012; 65(4): 437-44.
23 Blask DE, Sauer LA, Dauchy RT. Melatonin as a chronobiotic/anticancer agent: Cellular, biochemical, and molecular mechanisms of action and their implications for circadian-based cancer therapy. Curr Top Med Chem 2002; 2(2): 113-32.
24 Marciano DP, Chang MR, Corzo CA, Goswami D, Lam VQ, Pascal BD, *et al*. The therapeutic potential of nuclear receptor modulators for treatment of metabolic disorders: PPARgamma, RORs, and Rev-erbs. Cell Metab 2014; 19(2): 193-208.
25 Lardone PJ, Guerrero JM, Fernández-Santos JM, Rubio A, Martín-Lacave I, Carrillo-Vico A. Melatonin synthesized by T lymphocytes as a ligand of the retinoic acid-related orphan receptor. J Pineal Res 2011; 51(4):454-62.
26 Wang RX, Liu H, Xu L, Zhang H, Zhou RX. Involvement of nuclear receptor RZR/ROR gamma in melatonin-induced HIF-1alpha inactivation in SGC-7901 human gastric cancer cells. Oncol Rep 2015; 34(5): 2541-6.
27 Yeung HM, Hung MW, Lau CF, Fung ML. Cardioprotective effects of melatonin against myocardial injuries induced by chronic intermittent hypoxia in rats. J Pineal Res 2015; 58(1): 12-25.
28 Choi HS, Kang JW, Lee SM. Melatonin attenuates carbon tetrachloride-induced liver fibrosis via inhibition of necroptosis. Transl Res 2015; 166(3): 292-303.
29 Rodriguez C, Mayo JC, Sainz RM, Antolín I, Herrera F, Martín V, *et al*. Regulation of antioxidant enzymes: A significant role for melatonin. J Pineal Res 2004, 36(1): 1-9.
30 Hill SM, Blask DE, Xiang S, Yuan L, Mao L, Dauchy RT, *et al*. Melatonin and associated signaling pathways that control normal breast epithelium and breast cancer. J Mammary Gland Biol Neoplasia 2011; 16(3): 235-45.
31 Hill SM, Cheng C, Yuan L, Mao L, Jockers R, Dauchy B, *et al*. Declining melatonin levels and MT1 receptor expression in aging rats is associated with enhanced mammary tumor growth and decreased sensitivity to melatonin. Breast Cancer Res Treat 2011; 127(1): 91-8.
32 Leon J, Casado J, Carazo A, Sanjuan L, Mate A, Munoz de Rueda P, *et al*. Gender-related invasion differences associated with mRNA expression levels of melatonin membrane receptors in colorectal cancer. Mol Carcinog 2012; 51(8): 608-18.
33 Cohen M, Lippman M, Chabner B. Role of pineal gland in aetiology and treatment of breast cancer. Lancet 1978; 2(8094): 814-6.
34 Tamarkin L, Danforth D, Lichter A, DeMoss E, Cohen M, Chabner B, *et al*. Decreased nocturnal plasma melatonin peak in patients with estrogen receptor positive breast cancer. Science 1982; 216(4549): 1003-5.
35 Chottanapund S, van Duursen MB, Navasumrit P, Hunsonti P, Timtavorn S, Ruchirawat M, *et al*. Anti-aromatase effect of resveratrol and melatonin on hormonal positive breast cancer cells co-cultured with breast adipose fibroblasts. Toxicol In Vitro 2014; 28 (7): 1215-21.
36 Xu S, Pi H, Zhang L, Zhang N, Li Y, Zhang H, *et al*. Melatonin prevents abnormal mitochondrial dynamics resulting from the neurotoxicity of cadmium by blocking calcium-dependent translocation of Drp1 to the mitochondria. J Pineal Res 2016; 60(3): 291-302.
37 Proietti S, Cucina A, Reiter RJ, Bizzarri M. Molecular mechanisms of melatonin's inhibitory actions on breast cancers. Cell Mol Life Sci 2013; 70(12): 2139-57.
38 Blask DE, Hill SM, Dauchy RT, Xiang S, Yuan L, Duplessis T, *et al*. Circadian regulation of molecular, dietary, and metabolic signaling mechanisms of human breast cancer growth by the nocturnal melatonin signal and the consequences of its disruption by light at night. J Pineal Res 2011; 51(3): 259-69.
39 Gupta S, Haldar C, Ahmad R. Photoperiodic regulation of nuclear melatonin receptor RORalpha in lymphoid organs of a tropical rodent Funambulus pennanti: Role in seasonal oxidative stress. J Photochem Photobiol B 2015; 142: 141-53.
40 Um HJ, Kwon TK. Protective effect of melatonin on oxaliplatin-induced apoptosis through sustained Mcl-1 expression and anti-oxidant action in renal carcinoma Caki cells. J Pineal Res 2010; 49(3): 283-90.
41 Mao L, Yuan L, Slakey LM, Jones FE, Burow ME, Hill SM. Inhibition of breast cancer cell invasion by melatonin is mediated

through regulation of the p38 mitogen-activated protein kinase signaling pathway. Breast Cancer Res 2010; 12(6): R107.
42 Sotovega E, Meza I, Ramírezrodríguez G, Benitez-King G. Melatonin stimulates calmodulin phosphorylation by protein kinase C. J Pineal Res 2004; 37(2): 98-106.
43 del Rio B, Garcia Pedrero JM, Martinez-Campa C, Zuazua P, Lazo PS, Ramos S. Melatonin, an endogenous-specific inhibitor of estrogen receptor alpha via calmodulin. J Biol Chem 2004; 279(37): 38294-302.
44 Pohanka M. Impact of melatonin on immunity: A review. Cent Eur J Med 2013; 8(4): 369-76.
45 Calvo JR, Gonzalez-Yanes C, Maldonado MD. The role of melatonin in the cells of the innate immunity: A review. J Pineal Res 2013; 55(2): 103-20.
46 Blask DE, Dauchy RT, Sauer L, Davidson LK, Krause JA, Lynch DT, *et al*. Oral melatonin supplementation in rats and a human subject suppresses the growth activity of steroid receptor negative human breast cancer xenografts in female nude rats via an MT1 receptor-mediated suppression of signal tranduction and linoleic acid uptake and metabolism. Proc Amer Assoc Cancer Res 2005; 65(9 sup): 1358.
47 Garcia CP, Lamarque AL, Comba A, Berra MA, Silva RA, Labuckas DO, *et al*. Synergistic anti-tumor effects of melatonin and PUFAs from walnuts in a murine mammary adenocarcinoma model. Nutrition 2015; 31(4): 570-7.
48 Hardeland R. Melatonin in plants—diversity of levels and multiplicity of functions. Front Plant Sci 2016; 7: 198.