The Role of Melatonin in Improving Hypoxia in Malignant Tumor: A Mini-Review

Diani Kartini
Division of Oncology Surgery, Faculty of Medicine Universitas Indonesia, dr. Cipto Mangunkusumo General Hospital, Jakarta, d.kartini@gmail.com

Akmal Taher
Department of Urologi, Faculty of Medicine Universitas Indonesia, dr. Cipto Mangunkusumo General Hospital, Jakarta

Sonar S. Panigoro
Division of Oncology Surgery, Faculty of Medicine Universitas Indonesia, dr. Cipto Mangunkusumo General Hospital, Jakarta

Rianto Setiabudy
Department of Pharmacology and Therapeutics, Faculty of Medicine, dr. Cipto Mangunkusumo General Hospital, Jakarta

Sri W. Jusman
Department of Biochemistry and Molecular Genetics, Faculty of Medicine, dr. Cipto Mangunkusumo General Hospital, Jakarta

Follow this and additional works at: https://scholarhub.ui.ac.id/nrjs

Part of the Surgery Commons

See next page for additional authors

Recommended Citation

The Role of Melatonin in Improving Hypoxia in Malignant Tumor: A Mini-Review

Authors

Diani Kartini, Akmal Taher, Sonar S. Panigoro, Rianto Setiabudy, Sri W. Jusman, Sofia M. Haryana, Murdani Abdullah, and Primariadewi Rustamadji

The Role of Melatonin in Improving Hypoxia in Malignant Tumor:
A Mini–Review

Diani Kartini,[1] Akmal Taher,[2] Sonar S. Panigoro,[1] Rianto Setiabudy,[3] Sri W. Jusman,[4] Sofia M. Haryana,[5] Murdani Abdullah,[6] Primariadewi Rustamadji.[7]

1. Division of Oncology Surgery, Department of Surgery, 2. Department of Urology, 3. Department of Pharmacology and Therapeutics, 4. Department of Biochemistry and Molecular Genetics, Faculty of Medicine Universitas Indonesia, dr. Cipto Mangunkusumo General Hospital, Jakarta, 5. Department of Biomedical Science, Faculty of Medicine, Universitas Gajah Mada, Yogyakarta, 6. Division of Gastroenterology and Hepatology, Department of Internal Medicine, 7. Division of Anatomical Pathology, Department of Laboratory Medicine and Pathology, Faculty of Medicine Universitas Indonesia, dr. Cipto Mangunkusumo General Hospital, Jakarta.

Introduction

A most encountered problem in chemotherapy is tumor resistance towards chemotherapy drugs. Hypoxic condition in the microenvironment of the tumor is very likely caused by an imbalance of oxygen supply and consumption due to limited blood supply in rapidly proliferating tumor, resulting in restricted oxygen diffusion and expansion of the hypoxic area.[1] The main factor that plays a role in the hypoxic state is hypoxia–inducible factors (HIFs) protein. In hypoxic conditions, HIF–1α becomes stable and undergoes dimerization with β–subunit to form HIF–1, which in turn activates the transcription process of proteins required to adapt to hypoxia. Over expression of HIF–1α corresponds to chemotherapy and radiotherapy resistance, also mortality.[2–4]

Hypoxia also affects reactive oxygen species (ROS), where the escalation of ROS increases HIF–1α stability in inflammatory cells.[5] ROS deals with the body's antioxidant system, namely melatonin, which is an endogenous antioxidant. Systematic reviews and meta–analysis of 21 clinical trials reported a beneficial effect of melatonin with 10–40 mg/day in cancer patients, in conjunction with or without standard chemotherapy, given before or simultaneously with the chemotherapy.[6] Most clinical trials that employ oral melatonin of 20 mg/day (0.3 mg/kg/day), given at night. A minimal dose of melatonin required to exert its antitumor properties.[7]

Melatonin also has oncostatic properties through inducing apoptosis of cancer cells. Melatonin, given simultaneously with chemotherapy in solid tumors, increases chemotherapy response and survival. Nevertheless, there has not been an experimental study that can explain melatonin's influence in alleviating tumor clinical response to chemotherapy.

Literature searching carried out on four database sites (PubMed, ClinicalKey, ScienceDirect, and Wiley Online Library). The keywords used following medical subject headings, namely: 'melatonin', 'oral squamous cell carcinoma', 'melatonin, and oral squamous cell carcinoma, and solid tumor'. Fifty–eight studies included in this review.

Hypoxia in tumor

The hypoxic microenvironment caused by an imbalance in oxygen supply and consumption. The limited blood supply in the rapidly proliferating tumor impairs oxygen diffusion leading to hypoxia. Several factors may lead to hypoxia, namely pathological, physiological, emotional stress, and unfavorable environment. Oxygen deficiency occurs in all phases of growth and development of a solid tumor. Tumor hypoxia is mainly due to the lack of organization of blood vessels (new capillaries, angiogenesis) on the superficial layer of a solid tumor. However, nearly none reside inside, resulting in severe hypoxia in the center of the tumor mass. Hypoxic stress in solid tumor causes uncontrolled HIF and VEGF expression that triggers the proliferation and differentiation of vascular endothelial cell, followed with the formation of new blood vessels.[1,4]

In hypoxic state, cells may elicit response in three ways, namely: (a) inhibition of cell proliferation to restrain the number of cells that consume oxygen; (b) decrement of oxidative phosphorylation and increment of glycolysis let cellular oxygen consumption decreases, and (c) increased production of angiogenic factors to enhance oxygen delivery.[8]

Tissue hypoxia is a hallmark of nearly all solid tumors. It regulates different processes of cellular metabolism, cell survival and proliferation, angiogenesis, adhesion, and motility.[9] Adaptation to hypoxia is essential for tumor expansion, invasion, metastasis. Such an adaptation well recognized as treatment failure in clinical oncology.[10] Acute hypoxic stress triggers the development of aggressive cancer phenotype with rapid metastatic rate, resistance to therapeutic agents, and higher tumor recurrence. Prolonged lack of oxygen will generate chronic hypoxic stress and tumor necrosis.[1]

Hypoxia–Inducible Factor

HIF is known as a critical regulator in the cellular response to hypoxia that holds an important role in the development of the disease, especially cancer. The hypoxic tumors environment leads to the stabilization of HIF. HIF is crucial in critical biological aspects of cancer, namely in the angiogenesis process, stem cell maintenance, metabolic reprogramming, autocrine growth factor signaling, epithelial–mesenchymal transition, invasion, metastasis, also resistance to radiation therapy and chemotherapy.[11] HIF comprise of HIF–1α, HIF–1ß, HIF–2α, and HIF–3α. HIF–1α is the most important one of which, responsible for the activation of hypoxia–related transcriptional responses. HIF–1ß is a dimerization of HIF–1α, while HIF–2α and HIF–3α are isoforms. HIF–2α functions are similar to that of HIF–1α, which are to increase regulation of transcriptional activity while HIF–3α roles as a down–regulator in decreasing hypoxia response through HIF–1α mediator inhibitor.[3]

The oxygen concentration activity governs the HIF–1α and HIF–2α. In hypoxic conditions, these proteins degraded by the proteasome. Unlike subunit β, HIF–1α regulated intracellularly, which produced as a response to decreased oxygen level.[12] HIF is targeted for ubiquitination by oxygen–sensitive prolyl hydroxylases (PHD) and von Hippel–Lindau (VHL) tumor suppressor protein. In normoxic conditions, Factor Inhibiting HIF–1 (FIH) also causes inactivation due to HIF hydroxylation. In hypoxic conditions, there is a stabilization of HIF–1α and HIF–2α due to hydroxylation and HIF–1; all the mentioned factors undergo inhibition due to lack of oxygen. In hypoxic conditions, HIF–1α bound to its dimer, which is HIF–1β forming HIF–1, which later will translocate to the nucleus. Furthermore, HIF–1 bound to specific regulatory elements in target genes' promoters that induce its expression.[13]

Increased HIF–1α expression due to hypoxia and genetic mutation occurs in various cancers in humans, such as malignancy of the brain, breast, cervix, esophagus, oropharynx, and ovarium. Increased expression of HIF–1α and HIF–2α in the tumor is related to a more aggressive tumor phenotype and unfavorable survival prognosis, which reflects the impact of these factors on tumor development.[9,14] Increased expression also related to the resistance to chemotherapy, radiotherapy, and mortality.[4] HIF–1α plays an essential role in normal tumor growth and development. However, there are conflicting findings related to the theory. In the majority of cancer, HIF–1α expression might be correlated with poor prognosis ($p = 0.08$)[15] or can increase overall survival and disease–free survival. Thus, it can be concluded that the effect of HIF–1α expression depends explicitly on the type of cancer and the presence of genetic changes that affect the balance of pro– and antiapoptotic course.[16]

Hypoxia–Inducible Factor and Reactive Oxygen Species

ROS is a substance or molecule which has a single unpaired electron in its outer electron shell; therefore, ROS is highly reactive. ROS categorized into two groups: free radicals and nonradical. Free radicals are composed of superoxide ($O2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), nitric oxide ($NO^{\bullet}$), organic radicals

($R^{\bullet}$), peroxyl radicals ($ROO^{\bullet}$), alkoxyl radicals ($RO^{\bullet}$), thiyl radicals ($RS^{\bullet}$), sulfonyl radicals ($ROS^{\bullet}$), thiyl peroxyl radicals ($RSOO^{\bullet}$), and disulfides ($RSSR$). Non–radicals comprised of H_2O_2, singlet oxygen ($^1O_2$), ozone/trioxygen (O_3), organic hydroperoxides ($ROOH$), hypochloride ($HOCl$), peroxynitrite (ONO^-), nitrosoperoxycarbonate anion ($O^=NOOCO_2$), nitrocarbonate anion ($O2NOCO^{2-}$), dinitrogen dioxide (N_2O_2), nitronium (NO^{2+}), and carbonyl substance derived from highly reactive lipid or carbohydrate. Superoxide, H_2O_2, and radical hydroxyl are the types of ROS that more commonly studied in its relation to cancer.[17]

Several factors and ROS determine the number of free radicals. Endogenous sources of ROS include mitochondria, metabolic reaction through cytochrome P–450, peroxisome, and activation of inflammatory cells. There are abundant cellular systems that could produce ROS. Intracellular ROS primary resource is the respiratory chain in mitochondria, which utilizes 80–90% oxygen consumed by an individual and produces the majority of ROS in the body. Another source of ROS, particularly in the liver, is a group of enzymes, also known as cytochrome P–450 monooxygenase (mixed–function oxidase). ROS is also produced by several enzymes intracellularly, such as xanthine oxidase. In physiological condition, xanthine oxidase act as a dehydrogenase, which releases hydrogen from xanthine or hypoxanthine and attaches hydrogen to NAD, therefore, forming NADH. In conditions such as disturbance of tissue perfusion, xanthine dehydrogenase will be transformed into ROS–producing oxidase.[18–21]

Mitochondria play a role in the control of ATP production through electron chain transport, calcium homeostasis, apoptosis, and cell signaling, while ROS is involved in enhancing stabilization of HIF–1α in inflammatory cells.[22–24] Accumulation of HIF is an impact of increased ROS by NADPH oxidase.[5] In hypoxic conditions, ROS is also produced by mitochondria, which will activate ERK and p38 MAP kinase pathway. Activated ERK2 will phosphorylate HIF–1α and increase transcriptional activity (Figure 2.4).[25] Therefore, it is crucial to recognize mechanisms underlying HIF regulation and ROS production in cellular metabolism, oncogenesis, and stem cell biology.[5]
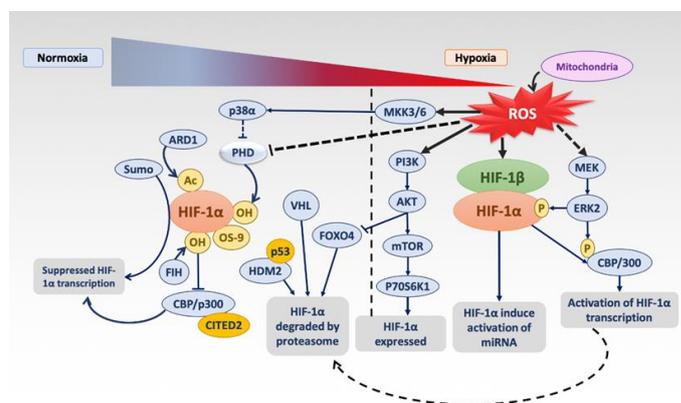

Figure 1. Primary Circuit of ROS signal regulation in normoxic and hypoxic condition.[25] Note. HIF–1α as a central node of the communication network. Straight line depicts known relationships, while the dashed line depicts relationships that require further investigation.

23

Effect of Hypoxia on Chemoresistance

Resistance to chemotherapy is the leading cause of therapy failure in inoperable and advanced–stage cancer. Causes for resistance are complex and can be divided into three group factors which are; pharmacokinetic resistance, intrinsic tumor cell resistance, and microenvironmental factors.[26,27] Hypoxia affects the microenvironment of stem cells and stemness phenotype; regulation of cell population's resistance to apoptosis; reduction of proapoptotic gene activity; autophagic induction and anaerobic metabolism activation. Intratumor hypoxia causes genomic instability through suppression of DNA repair pathway and ROS production; it also contributes to cellular resistance to radiotherapy and chemotherapy.[28]

Hypoxia–induced chemoresistance first reported in the 1980s, which elucidated that in chronic hypoxic conditions or severe hypoxia, tumor cells may become chemo resistant. It was also mentioned that hypoxia causes resistance of cancer cells to therapy through the regulation of various processes, namely: 1) Inducing cessation of cell cycle, through reduction of cell proliferation which protects the cell from external stressor; 2) Inhibiting apoptosis and cellular aging; 3) Controlling autophagy, p53, and mitochondrial activity.[29,30] Irrespective of several cellular adaptation mechanisms that are affected by hypoxia, reduced oxygenation in tumor tissue causes chemoresistance by affecting the drug delivery system and cellular uptake through related acidity and expression of drug efflux pump such as P–glycoprotein (P–gp) and decreasing oxygen reserve that needed for chemotherapy cytotoxicity.[31,32] Another critical point is that not only do cancer cells become resistant to a single drug, but also several others (multidrug resistance/MDR).

For growth, survival, and metastasis, cancer cells rely on their distance to the blood vessel. Reliance on tumor cell vascularization can be achieved through angiogenesis as growth and tumor progression depends on this process.[33] Tumor cells located 100–200 μm from blood vessels will become hypoxic, so to surpass the said limit, tumor cells will undergo angiogenesis that is governed by the balance of pro–angiogenic and anti–angiogenic molecules.[34] In the initial process of angiogenesis, small blood vessels rely on its major blood vessel; no solid tumor can grow >2 mm^3 except by synthesizing its new blood vessel.[33]

Hypoxia and expression of HIF–1 contributes in angiogenesis through several ways, namely activating angiogenic genes and its receptors through a transcription process (VEGF, Platelets–derived growth factor (PlGF), PDGFB, Angiopoietin 1 (ANGPT$_1$), and ANGPT$_2$); regulating proangiogenic chemokines and its receptors (SDF–1α, stromal cell–derived factor–1α, S1P, sphingosine–1–phosphate, and CXCR$_4$ receptor, C–X–C chemokine receptor type 4) thus facilitating endothelial progenitor cells round–up in hypoxic area and increasing proliferation and division of endothelial cell (regulating gene in cell cycle and DNA replication).[35]

Melatonin

Melatonin is a naturally produced hormone secreted by the pineal gland. In mammals, melatonin also synthesized by other organs, such as eyes, bone marrow, gastrointestinal tract, skin, and lymphocyte.[36] Unlike other antioxidants, melatonin can penetrate physiological barriers (including blood–brain barrier) and still achieves therapeutic concentration. Melatonin possesses two kinds of receptors that are bound to a G protein in the target cell, namely MT1 and MT2.[37] Radical–scavenging effect of melatonin is not achieved through those receptors. However, antioxidant enzyme activation involves these receptors in target cells.[38] Activation of these two receptors influences the regulation of cellular processes, namely neuron excitation, arterial vasoconstriction, cellular proliferation, immune response, also other metabolic and reproductive functions.[37] Other than MT1 and MT2, melatonin also interacts with the intracellular protein, namely ROR/RZR (located in the nucleus), quinone reductase 2 (known as MT$_3$). Retinoid–related orphan receptor (ROR)/retinoid Z receptor (RZR) is related to MT$_1$ and MT$_2$ in regulating gene expression.[39] Melatonin affects activity in almost every cell. It can be detected in membrane compartment, cytoplasm, mitochondria, and nucleus so that melatonin differs from any other hormones in the sense that not synthesized by a single organ, possesses nonspecific target organ, and has a dominant function of protecting cells from free radicals.[40,41] Melatonin also acts as a potent antioxidant, immunomodulator, anti–proliferation, oncostatic, and able to modulate endocrine glands.[42] Melatonin and its metabolite derivate is known as an antioxidant and potent radical scavenger due to its ability to 'digest' 10 ROS by forming antioxidant cascade so that it is far more effective than other antioxidants.[43]

Melatonin's oncostatic effect is related to its ability to inhibit tumor proliferation, induce apoptosis, prevent tumor cell invasion and metastasis, angiogenesis, and improve cellular immune activity.[42,44] Clinical trial results of melatonin on non–small cell lung cancer patients showed it could improve effectivity and reduce side effects of chemotherapy, improve survival rate, and improve patients' quality of life.[45]

With 2 mg and 4 mg orally, only 15% reaches up systemic circulation (bioavailability 15%), while the remaining 85% most likely undergo the first–pass metabolism.[46] This low bioavailability may be caused by low absorption from the digestive tract, liver first–pass metabolism, or both.[47] The central metabolism in the liver converts melatonin into its metabolite through hydroxylation and conjugation by sulfate or glucuronic acid; then, it excreted through urine.[48]

Melatonin is mainly metabolized in the liver by enzyme cytochrome P450 enzymes, which are CYP$_1$A$_2$, CYP$_1$A$_1$, and CYP$_1$B$_1$.[42] Melatonin is degraded by CYP$_1$A$_2$, thus competing with coffee and tea. T^1/$_2$ of caffeine during melatonin release is prolonged, and caffeine consumption delays attainment of peak melatonin concentration at nighttime.[49] Melatonin has a hydrophilic and lipophilic characteristic, its lipophilic property enables it to penetrate every biological membrane, including the blood–brain barrier.[50] In circulation, 70% of melatonin

binds to albumin while the rest (30%) diffuses freely into the tissue.[51] There had been numerous studies that explain the effect of melatonin in vitro or in vivo. Melatonin can reduce tumor cell growth and possesses anti carcinogenesis property.[52] Optimal dose for melatonin that commonly used in clinical trials is 20–40 mg.[6] Another effect of melatonin administration, in combination with adjuvant chemotherapy, is a significant reduction of adverse chemotherapy effects (nausea, vomiting, anemia, asthenia, leukopenia, and thrombocytopenia).[6]

Melatonin inhibits HIF–1α, a gene induced by HIF–1α and VEGF. In an in vitro study, melatonin inhibits expression of HIF–1α of prostate cancer cells, glioblastoma, VEGF expression in breast cancer cells, pancreatic cancer, liver cancer, and glioblastoma.[53–56] In Ewing sarcoma cell, tumor cell displays metabolic profile consistent with aerobic glycolysis such as an increase in glucose uptake, LDH activity, lactate production, and HIF–1α activation. Melatonin restores the metabolic profile of Ewing sarcoma–associated with cytotoxicity.[57]

Research on solid tumors conducted by Lissoni et al. .[58] concludes that the administration of melatonin in combination with chemotherapy can decrease the 1st year risk of death significantly (p <0.001) and increase response rate (p <0.001) compared to chemotherapy alone. The use of 20 mg melatonin per day on cancer patients in the long term does not show any significant toxicity effect.[58]

Melatonin administration thought to affect tumor microenvironment through lowering HIF–1 expression, resulting in decreased angiogenesis, increased apoptosis. Alleviation of hypoxia hoped to ameliorate tumor cells' response towards chemotherapy and radiotherapy.

Conclusion

Hypoxia in the microenvironment of solid tumors gives rise to a series of events, namely increased expression of HIF–1α, angiogenesis, and many more, which will cause chemoresistance and radio–resistance, resulting in treatment failure. Melatonin administration may improve hypoxia, thus ameliorate treatment response.

Reference

1. Li JZ, Gao W, Chan JY–W, Ho W–K, Wong T–S. Hypoxia in head and neck squamous cell carcinoma. ISRN Otolaryngol. 2012;2012:1–8.
2. Bar EE. Glioblastoma, cancer stem cells, and hypoxia. Brain Pathol. 2011;21(2):119–29.
3. Pérez–Sayáns M, Suárez–Peñaranda JM, Pilar GD, Barros–Angueira F, Gándara–Rey JM, García–García A. Hypoxia–inducible factors in OSCC. CancerLett. 2011;313(1):1–8.
4. Wang Y. Hypoxia–inducible factor: targeted anticancer prodrug. New Jersey: The state university of new jersey. 2007.
5. Saito S, Lin YC, Tsai MH, et al. Emerging roles of hypoxia–inducible factors and reactive oxygen species in cancer and pluripotent stem cells. Kaohsiung J Med Sci. 2015;31(6):279–86.

6. Seely D, Wu P, Fritz H, et al. Melatonin as adjuvant cancer care with and without chemotherapy: a systematic review and meta–analysis of randomized trials. Integr Cancer Ther. 2012;11(4):293–303.
7. Lissoni P. Melatonin in human cancer: therapeutic possibilities. Melatonin Melatonergic Drugs Clin Pract. 2014;9788132208:43–56.
8. Semenza GL. HIF–1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. J Clin Invest. 2013;123(9):3664–71.
9. Semenza G. Defining the role of hypoxia–inducible factor 1 in cancer biology and therapeutics. Oncogene. 2010;29(5):625–34.
10. Ivan M, Zhong X, Greco S, Martelli F. Emerging roles of non–coding RNAs in the hypoxic response. 2013;43–64.
11. Semenza GL. Hypoxia–inducible factors: mediators of cancer progression and targets for cancer therapy. Trends Pharmacol Sci. 2012;33(4):207–14.
12. Kusumbe AP, Bapat SA. Cancer stem cells and aneuploid populations within developing tumors are the significant determinants of tumor dormancy. Cancer Res. 2009;69(24):9245–53.
13. Brennan PA, Mackenzie N, Quintero M. Hypoxia–inducible factor 1α in oral cancer. Vol. 34, Journal of Oral Pathology and Medicine. 2005;385–9.
14. Bertout JA, Patel SA, Simon MC. The impact of O2 availability on human cancer. Nature Rev Cancer. 2008;8L:967–75.
15. Giatromanolaki A, Koukourakis MI, Sivridis E, Turley H, Talks K, Pezzella F, et al. Relation of hypoxia–inducible factor 1α and 2α inoperable non–small cell lung cancer to angiogenic/molecular profile of tumors and survival. Br J Cancer. 2001;85(6):881–90.
16. Beasley NJP, Leek R, Alam M, Turley H, Cox GJ, Gatter K, et al. Hypoxia–inducible factors HIF–1α and HIF–2α in head and neck cancer: relationship to tumor biology and treatment outcome in surgically resected patients. Cancer Res. 2002;62(9):2493–7.
17. Storz P, Liou GY. Reactive oxygen species in cancer. Free Radic Res. 2010;44(5):479–96.
18. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev. 1979;59(3):527–605.
19. Proulx M, du Souich P. Inflammation–induced decrease in hepatic cytochrome P450 in conscious rabbits is accompanied by an increase in hepatic oxidative stress. Res Commun Mol Pathol Pharmacol. 1995;87(2):221–36.
20. Sultatos LG. Effects of acute ethanol administration on the hepatic xanthine dehydrogenase/oxidase system in the rat. J Pharmacol Exp Ther. 1988;246(3):946–9.
21. Bhattacharya S. Reactive oxygen species and cellular defense system. In: Free Radicals in Human Health and Disease. 2015;17–29.
22. Dyall SD, Brown MT, Johnson PJ. Ancient invasions: from endosymbionts to organelles. Science. 2004;304:253–7.
23. Shatrov VA. Oxidized low–density lipoprotein (oxLDL) triggers hypoxia–inducible factor–1(HIF–1) accumulation via redox–dependent mechanisms. Blood. 2003;101(12):4847–9.
24. Yuan G, Nanduri J, Khan S, Semenza GL, Prabhakar NR. Induction of HIF–1α expression by intermittent hypoxia: involvement of NADPH oxidase, Ca2+ signaling, prolyl hydroxylases, and mTOR. J Cell Physiol. 2008;217(3):674–85.
25. Galanis A, Pappa A, Giannakakis A, Lanitis E, Dangaj D, Sandaltzopoulos R. Reactive oxygen species and HIF–1 signaling in cancer. Cancer Lett. 2008;266(1):12–20.

26. Gatti L, Zunino F. Overview of tumor cell chemoresistance mechanisms. In: Chemosensitivity. 2005;127–48.

27. Gottesman MM. Mechanisms of cancer drug resistance. Annu Rev Med. 2002;53(1):615–27.

28. Fer ND, Rapisarda A. Exploiting "Hif addiction" for cancer therapy. In 2013;329–41.

29. Rohwer N, Cramer T. Hypoxia–mediated drug resistance: novel insights on the functional interaction of HIFs and cell death pathways. Drug Resist Updat. 2011;14(3):191–201.

30. Das B, Tsuchida R, Malkin D, Koren G, Baruchel S, Yeger H. Hypoxia enhances tumor stemness by increasing the invasive and tumorigenic side population fraction. Stem Cells. 2008;26(7):1818–30.

31. Abraham J, Salama NN, Azab AK. The role of P–glycoprotein in drug resistance in multiple myeloma. Vol. 56, Leukemia and Lymphoma. 2015;26–33.

32. Cosse J–P, Michiels C. Tumour hypoxia affects the responsiveness of cancer cells to chemotherapy and promotes cancer progression. Anticancer Agents Med Chem. 2008;8(7):790–7.

33. Rak J. Angiogenesis. In: The basic science of oncology New York: Mc Graw hill. 2013;157–85.

34. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Vol. 407, Nature. 2000;249–57.

35. Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ, et al. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF–1. Blood. 2005;105(2):659–69.

36. Reiter RJ. Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. Endocr Rev. 1991;12(2):151–80.

37. Dubocovich ML, Markowska M. Functional MT 1 and MT 2 melatonin receptors in mammals. Vol. 27, Endocrine. 2005;101–10.

38. Pablos MI, Reiter RJ, Ortiz GG, Guerrero JM, Agapito MT, Chuang JI, et al. Rhythms of glutathione peroxidase and glutathione reductase in brain of chick and their inhibition by light. Neurochem Int. 1998;32(1):69–75.

39. Carrillo–Vico A, Guerrero JM, Lardone PJ, Reiter RJ. A review of the multiple actions of melatonin on the immune system. Vol. 27, Endocrine. 2005;189–200.

40. Srinivasan V, R. Pandi–Perumal S, Brzezinski A, P. Bhatnagar K, P. Cardinali D. Melatonin, Immune Function and Cancer. Recent Pat Endocr Metab Immune Drug Discov. 2012;5(2):109–23.

41. Tan DX, Manchester LC, Hardeland R, Lopez–Burillo S, Mayo JC, Sainz RM, et al. Melatonin: A hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. J Pineal Res. 2003;34(1):75–8.

42. Srinivasan V, Spence DW, Pandi–Perumal SR, Trakht I, Cardinali DP. Therapeutic actions of melatonin in cancer: Possible mechanisms. Vol. 7, Integrative Cancer Therapies. 2008;189–203.

43. Tan DX, Manchester LC, Esteban–Zubero E, Zhou Z, Reiter RJ. Melatonin as a potent and inducible endogenous antioxidant: Synthesis and metabolism. Vol. 20, Molecules. 2015;18886–906.

44. Bojková B, Kubatka P, Qaradakhi T, Zulli A, Kajo K. Melatonin may increase anticancer potential of pleiotropic drugs. Int J Mol Sci. 2018;19(12):3910.

45. Lissoni P, Chilelli M, Villa S, Cerizza L, Tancini G. Five years survival in metastatic non–small cell lung cancer patients treated with chemotherapy alone or chemotherapy and melatonin: a randomized trial. J Pineal Res. 2003;35(1):12–5.

46. Demuro RL, Nafziger AN, Blask DE, Menhinick AM, Bertino JS. The absolute bioavailability of oral melatonin. J Clin Pharmacol. 2000;40(7):781–4.

47. Di W–L, Kadva A, Johnston A, Silman R. Variable bioavailability of oral melatonin. N Engl J Med. 2002;336(14):1028–9.

48. Brzezinski A. Melatonin in humans. J Physiol Pharmacol. 2006;57(SUPPL. 5):19–39.

49. Härtter S, Nordmark A, Rose DM, Bertilsson L, Tybring G, Laine K. Effects of caffeine intake on the pharmacokinetics of melatonin, a probe drug for CYPIA2 activity. Br J Clin Pharmacol. 2003;56(6):679–82.

50. Reiter RJ, Guerrero JM, Escames G, Pappolla MA, Acuña–Castroviejo D. Prophylactic actions of melatonin in oxidative neurotoxicity. Ann N Y Acad Sci. 1997;825:70–8.

51. Czesnikiewicz–Guzik M, Konturek SJ, Loster B, Wisniewska G, Majewski S. Melatonin and its role in oxidative stress–related diseases of the oral cavity. J Physiol Pharmacol. 2007;58(SUPPL. 3):5–19.

52. Bartsch C, Bartsch H. The anti–tumor activity of pineal melatonin and cancer enhancing lifestyles in industrialized societies. Cancer Causes Control. 2006;17(4):559–71.

53. Lv D, Cui PL, Yao SW, Xu YQ, Yang ZX. Melatonin inhibits the expression of vascular endothelial growth factor in pancreatic cancer cells. Chinese J Cancer Res. 2012;24(4):310–6.

54. Park JW, Hwang MS, Suh S Il, Baek WK. Melatonin down–regulates HIF–1α expression through inhibition of protein translation in prostate cancer cells. J Pineal Res. 2009;46(4):415–21.

55. Zhang Y, Liu Q, Wang F, Ling EA, Liu S, Wang L, et al. Melatonin antagonizes hypoxia–mediated glioblastoma cell migration and invasion via inhibition of HIF–1α. J Pineal Res. 2013;55(2):121–30.

56. Alvarez–García V, González A, Alonso–González C, Martínez–Campa C, Cos S. Regulation of vascular endothelial growth factor by melatonin in human breast cancer cells. J Pineal Res. 2013;54(4):373–80.

57. Sanchez–Sanchez AM, Antolin I, Puente–Moncada N, Suarez S, Gomez–Lobo M, Rodriguez C, et al. Melatonin cytotoxicity is associated to Warburg effect inhibition in Ewing sarcoma cells. PLoS One. 2015;10(8):1–15.

58. Lissoni P, Barni S, Mandalà M, Ardizzoia A, Paolorossi F, Vaghi M, et al. Decreased toxicity and increased efficacy of cancer chemotherapy using the pineal hormone melatonin in metastatic solid tumour patients with poor clinical status. Eur J Cancer. 1999;35(12):1688–92.