褪黑素介导小鼠肝H22肿瘤细胞自噬作用的研究

《现代肿瘤医学》[ISSN:1672-4992/CN:61-1415/R] 期数: 2019年05期 页码: 733-738 栏目: 论著（基础研究） 出版日期: 2019-02-01

Title: Study on melatonin-mediated autophagy of tumor cells in H22 tumor BALB/c mice

作者: 刘 畅; 张宗恺; 贾 佳; 陈点点; 阮新建; 刘彦芳; 张 侠
中国人民解放军总医院第七医学中心肿瘤科，北京 100700

Author(s): Liu Chang; Zhang Zongkai; Jia Jia; Chen Diandian; Ruan Xinjian; Liu Yanfang; Zhang Xia
Department of Oncology，the Seventh Medical Center of PLA General Hospital，Beijing 100700，China.

关键词: 褪黑素; 细胞自噬; 细胞信号转导; 自噬因子; 肿瘤细胞

Keywords: melatonin; autophagy; cell-cell signaling; autophagy factor; tumor cells

分类号: R73-3

DOI: 10.3969/j.issn.1672-4992.2019.05.005

文献标识码: A

摘要: 目的：探讨褪黑素（melatonin，MLT）对小鼠肿瘤细胞自噬信号通路的影响，从而阐述褪黑素发挥抗肿瘤效果的机制。方法：构建H22肝癌模型小鼠30只，每组10只，分别为生理盐水组、10 mg/kg褪黑素组、20 mg/kg褪黑素组。将小鼠肿瘤组织制备成切片后于光学显微镜和电镜下观察切片。利用荧光免疫组化法检测每个视野中的阳性细胞数平均百分比，以该切片阳性细胞百分比为基础进行计分。通过蛋白印迹试验对蛋白进行定性及定量测定，最后利用荧光显微镜下观察细胞内的绿色荧光，记录图像，分析计数LC3阳性细胞与总细胞数的比例，反映细胞发生自噬的程度。结果：MLT处理的动物其自噬形态较生理盐水组明显增多。随机成像后MLT处理组自噬液泡总数明显高于生理盐水组（P<0.05）。经MLT处理的H22荷瘤小鼠的肿瘤组织中Beclin-1、LC3-Ⅱ表达水平明显高于生理盐水组（P<0.05）。MLT处理的H22荷瘤小鼠肿瘤组织中，LC3亮点有所增强。MLT处理的H22荷瘤小鼠肿瘤组织中Akt和mTOR磷酸化水平较生理盐水组明显降低（P<0.05）。结论：褪黑素在H22荷瘤小鼠中诱导自噬，褪黑素能够诱导自噬过程标志蛋白Beclin-1和LC3的表达，并且能够抑制H22荷瘤小鼠中的Akt/mTOR信号通路。

Abstract: Objective：To discuss the effect of melatonin （MLT） on autophagy signal pathway in mice tumor cells and the mechanism of melatonin to play an antitumor effect.Methods：30 models of H22 tumor BALB/c mice were established with 10 rats in each group，including the normal saline group，the 10 mg/kg and the 20 mg/kg melatonin group.The tumor tissue of mice was sliced and sectioned by optical microscope and electron microscope.The average percentage of the number of positive cells in each field of vision was detected by the method of immunofluorescence，which was based on the percentage of the positive cells of the slice.The protein was qualitatively and quantitatively determined by Western blotting.At last，the green fluorescence in cells was observed under fluorescence microscope，and the images were recorded.The ratio of LC3 positive cells to total cells was counted and analyzed，which reflects the degree of autophagy.Results：The autophagic morphology of the MLT treated animals was significantly higher than that in the normal saline group.The total number of autophagic vacuoles in the MLT treatment group was significantly higher than that of the normal saline group after random imaging （P<0.05）.In the tumor tissue of H22 bearing mice treated with melatonin，expression levels of Beclin-1 and LC3-Ⅱ were significantly higher than normal saline group （P<0.05） and the bright spot of LC3 was enhanced.The level of phosphorylation of Akt and mTOR in the tumor tissues of melatonin treated H22 tumor mice was significantly lower than that in the normal saline group （P<0.05）.Conclusion：Melatonin induces autophagy in H22 tumor mice and induces the expression of autophagy Beclin-1 and LC-3.MLT can also inhibit the Akt/mTOR signaling pathway in H22 tumor bearing mice.

参考文献/REFERENCES

[1] Tuli HS，Kashyap D，Bedi SK，et al.Molecular aspects of metal oxide nanoparticle (MO-NPs) mediated pharmacological effects [J] .Life Sci，2015，143：71-79.
[2]Leja-Szpak A，Pierzchalski P，Goralska M，et al.Kynuramines induce overexpression of heat shock proteins

in pancreatic cancer cells via 5-hydroxytryptamine and MT1/MT2 receptors [J] .J Physiol Pharmacol, 2015, 66(5): 711-718.
[3]Manchester LC, Coto-Montes A, Boga JA, et al.Melatonin: An ancient molecule that makes oxygen metabolically tolerable [J] .J Pineal Res, 2015, 59(4): 403-419.
[4]Zhang JJ, Li Y, Zhou T, et al.Bioactivities and health benefits of mushrooms mainly from China [J] .Molecules, 2016, 21(7): 938.
[5]Li Y, Zhang JJ, Xu DP, et al.Bioactivities and health benefits of wild fruits [J] .Int J Mol Sci, 2016, 17(8): 1258.
[6]Zhou Y, Zheng J, Li Y, et al.Natural polyphenols for prevention and treatment of cancer [J] .Nutrients, 2016, 8(8): 515.
[7]Zheng J, Zhou Y, Li Y, et al.Spices for prevention and treatment of cancers [J] .Nutrients, 2016, 8(8): 495.
[8]Xu DP, Zheng J, Zhou Y, et al.Extraction of natural antioxidants from the thelephora ganbajun mushroom by an ultrasound-assisted extraction technique and evaluation of antiproliferative activity of the extract against human cancer cells [J] .Int J Mol Sci, 2016, 17(10): 1664.
[9]Zhang YJ, Gan RY, Li S, et al.Antioxidant phytochemicals for the prevention and treatment of chronic diseases [J] .Molecules, 2015, 20(12): 21138-21156.
[10]Li AN, Li S, Zhang YJ, et al.Resources and biological activities of natural polyphenols [J] .Nutrients, 2014, 6(12): 6020-6047.
[11]Li F, Li S, Li HB, et al.Antiproliferative activity of peels, pulps and seeds of 61 fruits [J] .Journal of Functional Foods, 2013, 5(3): 1298-1309.
[12]Houghton PJ, Huang S.mTOR as a target for cancer therapy [J] .Curr Top Microbiol Immunol, 2004, 279: 339-459.
[13]Easton JB, Houghton PJ.mTOR and cancer therapy [J] .Oncogene, 2006, 25(48): 6436-6346.
[14]Serrano-Puebla A, Boya P.Lysosomal membrane permeabilization in cell death: New evidence and implications for health and disease [J] .Ann N Y Acad Sci, 2016, 1371(1): 30-44.
[15]Mizushima N, Levine B, Cuervo AM, et al.Autophagy fights disease through cellular self-digestion [J] .Nature, 2008, 451(7182): 1069-1075.
[16]Chen N, Karantza-Wadsworth V.Role and regulation of autophagy in cancer [J] .Biochimica Et Biophysica Acta Molecular Cell Research, 2009, 1793(9): 1516-1523.
[17]Mathew R, Karantzawadsworth V, White E.Role of autophagy in cancer [J] .Medical Recapitulate, 2010, 7(12): 961.
[18]Jung CH, Ro SH, Cao J,et al.mTOR regulation of autophagy [J] .FEBS Lett, 2010, 584(7): 1287-1295.
[19]Kroemer G, Marino G, Levine B.Autophagy and the integrated stress response [J] .Mol Cell, 2010, 40(2): 280-293.
[20]LoPiccolo J, Blumenthal GM, Bernstein WB, et al.Targeting the PI3K/Akt/mTOR pathway: Effective combinations and clinical considerations [J] .Drug Resist Updat, 2008, 11(1-2): 32-50.
[21]Yen WL, Klionsky DJ.How to live long and prosper: Autophagy, mitochondria, and aging [J] .Physiology (Bethesda), 2008, 23: 248-262.
[22]Nopparat C, Porter JE, Ebadi M, et al.The mechanism for the neuroprotective effect of melatonin against methamphetamine-induced autophagy [J] .J Pineal Res, 2010, 49(4): 382-389.
[23]Chen HD, Kao CY, Liu BY, et al.HLH-30/TFEB-mediated autophagy functions in a cell-autonomous manner for epithelium intrinsic cellular defense against bacterial pore-forming toxin in C.elegans [J] .Autophagy, 2017, 13(2): 371-385.
[24]Furusawa E, Hirazumi A, Story S, et al.Antitumour potential of a polysaccharide-rich substance from the fruit juice of Morinda citrifolia (Noni) on sarcoma 180 ascites tumour in mice [J] .Phytother Res, 2003, 17(10): 1158-1164.
[25]Eom JM, Seo MJ, Baek JY, et al.Alpha-eleostearic acid induces autophagy-dependent cell death through targeting AKT/mTOR and ERK1/2 signal together with the generation of reactive oxygen species [J] .Biochem Biophys Res Commun, 2010, 391(1): 903-908.
[26]Martin V, Herrera F, Carrera-Gonzalez P, et al.Intracellular signaling pathways involved in the cell growth inhibition of glioma cells by melatonin [J] .Cancer Res, 2006, 66(2): 1081-1088.
[27]Tengattini S, Reiter RJ, Tan DX, et al.Cardiovascular diseases: Protective effects of melatonin [J] .J Pineal Res, 2008, 44(1): 16-25.
[28]Otalora BB, Madrid JA, Alvarez N, et al.Effects of exogenous melatonin and circadian synchronization on tumor progression in melanoma-bearing C57BL6 mice [J] .J Pineal Res, 2008, 44(3): 307-315.
[29]Trubiani O, Recchioni R, Moroni F, et al.Melatonin provokes cell death in human B-lymphoma cells by mitochondrial-dependent apoptotic pathway activation [J] .J Pineal Res, 2005, 39(4): 425-431.
[30]Garcia JJ, Pinol-Ripoll G, Martinez-Ballarin E, et al.Melatonin reduces membrane rigidity and oxidative damage in the brain of SAMP8 mice [J] .Neurobiol Aging, 2011, 32(11): 2045-2054.
[31]Garcia-Santos G, Antolin I, Herrera F, et al.Melatonin induces apoptosis in human neuroblastoma cancer cells [J] .J Pineal Res, 2006, 41(2): 130-135.
[32]Kimball SR, Abbas A, Jefferson LS.Melatonin represses oxidative stress-induced activation of the MAP kinase and mTOR signaling pathways in H4IIE hepatoma cells through inhibition of Ras [J] .J Pineal Res, 2008, 44(4): 379-386.

[33]Proietti S，Cucina A，D'Anselmi F，et al.Melatonin and vitamin D3 synergistically down-regulate Akt and MDM2 leading to TGFbeta-1-dependent growth inhibition of breast cancer cells［J］.J Pineal Res，2011，50(2)：150-158.
[34]Casado-Zapico S，Rodriguez-Blanco J，Garcia-Santos G，et al.Synergistic antitumor effect of melatonin with several chemotherapeutic drugs on human ewing sarcoma cancer cells：Potentiation of the extrinsic apoptotic pathway［J］.J Pineal Res，2010，48(1)：72-80.
[35]Fang J，Ding M，Yang L，et al.PI3K/PTEN/AKT signaling regulates prostate tumor angiogenesis［J］.Cell Signal，2007，19(12)：2487-2497.
[36]Liu LZ，Hu XW，Xia C，et al.Reactive oxygen species regulate epidermal growth factor-induced vascular endothelial growth factor and hypoxia-inducible factor-1alpha expression through activation of AKT and P70S6K1 in human ovarian cancer cells［J］.Free Radic Biol Med，2006，41(10)：1521-1533.
[37]Carnesecchi S，Carpentier JL，Foti M，et al.Insulin-induced vascular endothelial growth factor expression is mediated by the NADPH oxidase NOX3［J］.Exp Cell Res，2006，312(17)：3413-3424.
[38]Petiot A，Ogier-Denis E，Blommaart EF，et al.Distinct classes of phosphatidylinositol 3'-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells［J］.J Biol Chem，2000，275(2)：992-998.
[39]Lum JJ，Bauer DE，Kong M，et al.Growth factor regulation of autophagy and cell survival in the absence of apoptosis［J］.Cell，2005，120(2)：237-248.
[40]Granato M，Gilardini MM，Romeo MA，et al.Metformin triggers apoptosis in PEL cells and alters bortezomib-induced unfolded protein response increasing its cytotoxicity and inhibiting KSHV lytic cycle activation［J］.Cell Signal，2017，40：239-247.
[41]Sun T，Li X，Zhang P，et al.Acetylation of Beclin 1 inhibits autophagosome maturation and promotes tumour growth［J］.Nat Commun，2015，6：7215.

备注/Memo: National Natural Science Foundation of China(No.81073087）；国家自然科学基金（编号：81073087）

更新日期/Last Update: 2019-02-01