

# EBSS饥饿人鼻咽癌CNE-2细胞株诱导自噬的机制研究

《现代肿瘤医学》[ISSN:1672-4992/CN:61-1415/R] 期数: 2019年01期 页码: 30-35 栏目: 论著(基础研究) 出版日期: 2018-11-30

**Title:** Research of EBSS starvation induced autophagy and its mechanism in CNE-2 cells

**作者:** 刘芮吟; 吴梦鸽; 曾汶; 张睿; 李诗琴; 费继敏

昆明医科大学第三附属医院, 云南省肿瘤医院头颈外科, 云南 昆明 650118

**Author(s):** Liu Ruiyin; Wu Mengge; Zeng Wen; Zhang Rui; Li Shiqin; Fei Jimin

Department of Head and Neck, Third Affiliated Hospital of Kunming Medical, Yunnan Kunming 650118, China.

**关键词:** 鼻咽癌; 自噬; 饥饿; LC3; LKB1; Beclin1

**Keywords:** nasopharyngeal neoplasms; autophagy; hunger; LC3; LKB1; Beclin 1

**分类号:** R739.6

**DOI:** 10.3969/j.issn.1672-4992.2019.01.008

**文献标识码:** A

**摘要:** 目的: 观察饥饿状态下永生生化正常鼻咽上皮NP69细胞、鼻咽癌CNE-2细胞的自噬变化及自噬与凋亡的相互作用, 为Baf-A1在鼻咽癌中的应用前景提供证据。方法: EBSS溶液替代DMEM溶液在NP69和CNE-2细胞中制造体外饥饿环境, Baf-A1抑制细胞自噬。应用Western blot及透射电镜检测细胞自噬, MTT检测细胞存活率, qRT-PCR检测mRNA表达。结果: 与NP69细胞相比, EBSS制造的饥饿环境显著促进CNE-2细胞发生自噬, 处理24时后出现大量细胞凋亡, 100 nmol/L Baf-A1抑制细胞自噬可延缓饥饿诱导的细胞凋亡。qRT-PCR检测结果: EBSS作用下, LKB1、AMPK、ULK1、Beclin1、ATG5 mRNA水平均显著上升( $P < 0.05$ )。结论: EBSS通过LKB1/AMPK/mTOR通路显著诱导CNE-2细胞自噬的发生。

**Abstract:** Objective: To investigate starvation induced autophagy and mechanism in normal nasopharyngeal cell line NP69 and nasopharyngeal carcinoma CNE-2 cell, to provide evidence for the application of Baf-A1 in nasopharyngeal carcinoma. Methods: NP69 and CNE-2 cell were starvation induced by EBSS medium instead of DMEM medium, Baf-A1 inhibit autophagy of CNE-2 cell. Western blot were used to detect the special markers of autophagic microtubule-associated protein 1 light chain 3(LC3), and transmission electron microscope(TEM) was used to observe the autophagic body. In condition, cell proliferation and apoptosis induced by nutrient depletion was measured by MTT assay. qRT-PCR analysis of the mRNA expression of autophagy related genes. Results: Compared with NP69, CNE-2 cell autophagy activity was significantly promoted in starvation condition induced by EBSS solution. The cell apoptosis was delayed by 100 nmol/L Baf-A1 which inhibit autophagy of CNE-2 cell. Meanwhile, the results of qRT-PCR demonstrated EBSS significantly promoted mRNA levels of LKB1, AMPK, ULK1, Beclin1, ATG5 in CNE-2 cells ( $P < 0.05$ ). Conclusion: The starvation condition may induce autophagy of CNE-2 cells by LKB1/AMPK/mTOR pathway.

## 参考文献/REFERENCES

- [1] LA Torre, F Bray, RL Siegel, et al. Global cancer statistics, 2012 [J]. CA: A Cancer Journal for Clinicians, 2015, 65(2): 87-108.
- [2] BE Fitzwalter, A Thorburn. Recent insights into cell death and autophagy [J]. Febs Journal, 2015, 282(22): 4279-4288.
- [3] S Piya, M Andreeff, G Borthakur. Targeting autophagy to overcome chemoresistance in acute myelogenous leukemia [J]. Autophagy, 2017, 13(1): 214-215.
- [4] Q Tan, M Wang, M Yu, et al. Role of autophagy as a survival mechanism for hypoxic cells in tumors [J]. Neoplasia, 2016, 18(6): 347.
- [5] E White. Deconvoluting the context-dependent role for autophagy in cancer [J]. Nature Reviews Cancer, 2012, 12(6): 401.
- [6] Y Wang, W Yin, X Zhu. Blocked autophagy enhances radiosensitivity of nasopharyngeal carcinoma cell line CNE-2 in vitro [J]. Acta oto-laryngologica, 2014, 134(1): 105.
- [7] SE Chow, YW Chen, CA Liang, et al. Wogonin induces cross-regulation between autophagy and apoptosis via a variety of Akt pathway in human nasopharyngeal carcinoma cells [J]. Journal of Cellular Biochemistry, 2012, 107(1): 1-10.

2012, 113(11): 3476-3485.

[8]G Mariño, M Nisosantano, EH Baehrecke, et al. Self-consumption: The interplay of autophagy and apoptosis [J]. *Nat Rev Mol Cell Biol*, 2014, 15(2): 81-94.

[9]SY Park, EG Sun, Y Lee, et al. Autophagy induction plays a protective role against hypoxic stress in human dental pulp cells [J]. *Journal of Cellular Biochemistry*, 2018, 119(2):1992-2002.

[10]Y Cai, Z Wan, T Sun, et al. Diarylquinoline compounds induce autophagy-associated cell death by inhibiting the Akt pathway and increasing reactive oxygen species in human nasopharyngeal carcinoma cells [J]. *Oncology Reports*, 2013, 29(3): 983-992.

[11]G Kroemer, G Mariño, B Levine. Autophagy and the integrated stress response [J]. *Molecular Cell*, 2010, 40(2): 280.

[12]ET Chang, HO Adami. The enigmatic epidemiology of nasopharyngeal carcinoma [J]. *Cancer Epidemiol Biomarkers Prev*, 2006, 15(10): 1765-1777.

[13]JD Xie, F Chen, YX He, et al. Old age at diagnosis increases risk of tumor progression in nasopharyngeal cancer [J]. *Oncotarget*, 2016, 7(40): 66170-66181.

[14]S Ladoire, L Senovilla, C Dalban, et al. Combined evaluation of LC3B puncta and HMGB1 expression predicts residual risk of relapse after adjuvant chemotherapy in breast cancer [J]. *Autophagy*, 2015, 11(10): 1878-1890.

[15]A Yamamoto, Y Tagawa, T Yoshimori, et al. Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells [J]. *Cell Structure & Function*, 1998, 23(1): 33.

[16]RC Russell, Y Tian, H Yuan, et al. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase [J]. *Nature Cell Biology*, 2013, 15(7): 741-750.

[17]HG Xia, L Zhang, G Chen, et al. Control of basal autophagy by calpain1 mediated cleavage of ATG5 [J]. *Autophagy*, 2010, 6(1): 61.

[18]J Liang, SH Shao, ZX Xu, et al. The energy sensing LKB1 [ndash] AMPK pathway regulates p27kip1 phosphorylation mediating the decision to enter autophagy or apoptosis [J]. *Nature Cell Biology*, 2007, 9(2): 218.

[19]GH Li, XL Lin, H Zhang, et al. Ox-Lp(a) transiently induces HUVEC autophagy via an ROS-dependent PAMP-1-LKB1-AMPK-mTOR pathway [J]. *Atherosclerosis*, 2015, 243(1): 223-235.

[20]A Sun, C Li, R Chen, et al. Gsk-3B controls autophagy by modulating LKB1-AMPK pathway in prostate cancer cells [J]. *Prostate*, 2016, 76(2): 172.

[21]M Yang, H Zhao, G Li, et al. Autophagy-based survival prognosis in human colorectal carcinoma [J]. *Oncotarget*, 2015, 6(9): 7084.

[22]Q Cui, S Tashiro, S Onodera, et al. Autophagy preceded apoptosis in oridonin-treated human breast cancer MCF-7 cells [J]. *Biological & Pharmaceutical Bulletin*, 2007, 30(5): 859.

[23]L Song, H Liu, L Ma, et al. Inhibition of autophagy by 3-MA enhances endoplasmic reticulum stress-induced apoptosis in human nasopharyngeal carcinoma cells [J]. *Oncology Letters*, 2013, 6(4): 1031.

[24]A Eisenberglerner, S Bialik, HU Simon, et al. Life and death partners: Apoptosis, autophagy and the cross-talk between them [J]. *Cell Death & Differentiation*, 2009, 16(7): 966.

[25]M Lippai, Z Szatmári. Autophagy-from molecular mechanisms to clinical relevance [J]. *Cell Biology & Toxicology*, 2017, 33(2): 1-24.

[26]R Wang. Two's company, three's a crowd: Can H2S be the third endogenous gaseous transmitter [J] ? *Faseb Journal Official Publication of the Federation of American Societies for Experimental Biology*, 2002, 16(13): 1792.

---

**备注/Memo:** 云南省卫生高层次人才基金资助项目(编号: D-201659)

---

更新日期/Last Update: 2018-11-30