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自噬在声动力疗法抑制 C6 胶质瘤细胞增殖中的作用 *

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摘要 目的：探讨自噬在血卟啉单甲醚(Hematoporphyrin monomethyl ether，HMME)介导的声动力疗法(Sonodynamic therapy，SDT)抑制 C6 胶质瘤细胞增殖中的作用。**方法：**选取对数期生长的 C6 胶质瘤细胞并随机分为四组：对照组（未予处理）、超声组（单独超声照射）、HMME 组（单独加入 HMME）、SDT 组(超声照射 +HMME)。透射电镜观察 SDT 处理的 C6 胶质瘤细胞中自噬体数量的改变。应用 qRT-PCR 和免疫印迹分析 SDT 处理对 C6 胶质瘤细胞中的 LC3、Beclin1、Bcl-2 mRNA 及蛋白表达水平的影响。MTT 检测 C6 胶质瘤细胞的活力变化。**结果：**透射电子显微镜显示 SDT 组自噬体数量较对照组明显增多。SDT 组 C6 胶质瘤细胞中微管相关蛋白 1 轻链 3 (Microtubule associated protein 1 light chain 3, LC3)、Beclin1 mRNA 和蛋白水平高于对照组，B 细胞淋巴瘤 -2 (B cell lymphoma-2, Bcl-2) mRNA 和蛋白水平低于对照组。与对照组相比，SDT 组 C6 胶质瘤细胞存活率从 0 h 至 6 h 逐渐下降，从 12 h 至 72 h 逐渐升高。3- 甲基腺嘌呤(3-Methyladenine,3-MA)+SDT、氯喹(Chloroquine,CQ)+SDT 处理后 C6 胶质瘤细胞存活率较 SDT 组明显降低。**结论：**SDT 可能通过诱导自噬抑制 C6 胶质瘤细胞增殖。

关键词：自噬；声动力疗法；C6 胶质瘤细胞；微管相关蛋白 1 轻链 3；Beclin1

中图分类号：R-33；R739.4 **文献标识码：**A **文章编号：**1673-6273(2019)19-3616-05

The Role of Autophagy in the Inhibition of Proliferation of C6 Glioma Cells by Sonodynamic Therapy*

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ABSTRACT Objective: To investigate the role of autophagy in Hematoporphyrin monomethyl ether (HMME)-mediated Sonodynamic therapy (SDT) in inhibiting the proliferation of C6 glioma cells. **Methods:** C6 glioma cells grown in log phase were randomly divided into four groups: control group (not treated), ultrasound group (individual ultrasound irradiation), HMME group (HMME alone), SDT group (ultrasound irradiation + HMME). The number of autophagic vacuoles in SDT treated C6 glioma cells was observed by transmission electron microscopy. The effects of SDT treatment on the expression of LC3, Beclin1, Bcl-2 in C6 glioma cells were analyzed by qRT-PCR and Western blot. MTT was used to detect the viability of C6 glioma cells. **Results:** Transmission electron microscopy analysis demonstrated that the number of autophagy in SDT group was significantly higher than that in the control group. The mRNA and protein levels of microtubule associated protein 1 light chain 3 (LC3) and Beclin1in C6 glioma cells of SDT group were higher than those of the control group, and the levels of B cell lymphoma-2(Bcl-2) mRNA and protein were lower than those of the control group. Compared with the control group, the viability of C6 glioma cells in SDT group decreased from 0 h to 6 h, and increased from 12 h to 72 h. The viability of C6 glioma cells after 3-methyladenine (3-MA)+SDT and Chloroquine (CQ)+SDT treatment was significantly lower than that of SDT group. **Conclusion:** SDT may inhibit the proliferation of C6 glioma cells by inducing autophagy.

Key words: Autophagy; Sonodynamic therapy; C6 glioma cells; Microtubule-associated protein 1 light chain 3; Beclin 1

Chinese Library Classification(CLC): R-33; R739.4 Document code: A

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前言

胶质瘤是成人脑肿瘤中最致命的恶性肿瘤[1,2]，治疗后易复发。SDT 依赖于超声和声敏剂的协同作用，是具有无创性、更深的穿透性的方法[3,4]。SDT 可通过诱导细胞凋亡、ROS 产生、细胞内钙释放、钙超载及坏死而杀死胶质瘤细胞和抑制胶质瘤细

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胞生长[5-7]。但是否有其他机制参与 SDT 对 C6 胶质瘤细胞增殖的抑制作用仍不清楚。

自噬是一种降解和回收细胞成分的基本过程[8,9],虽然自噬的主要作用是维持细胞的存活[10],但胶质瘤中过度自噬可诱导 II 型程序性细胞死亡[11]。研究表明凋亡(I 型程序性细胞死亡)可与自噬相互作用[12,13],自噬已作为目前胶质瘤治疗的重要方向[14]。SDT 可诱导小鼠乳房癌 4T1 细胞系自噬[15],但其是否影响 C6 胶质瘤细胞的自噬仍不清楚。本研究重要探讨了自噬在 SDT 对 C6 胶质瘤细胞增殖影响中的作用，以期为胶质瘤的治疗提供更多的参考。

1 材料与方法

1.1 材料

3-MA、CQ(Sigma);MTT(上海碧云天生物技术有限公司);兔源 LC3B 抗体(Sigma);RIPA 裂解液(杭州昊鑫生物科技股份有限公司); RPMI-1640、0.25 %Typsin-EDTA (Gibco);胎牛血清(Invitrogen);兔源 Beclin 1 抗体(Santa Cruz);1 % 双抗链霉素和青霉素(Beyotime);C6 细胞(哈尔滨医科大学脑科研究所);Abstract H-7650 电子显微镜(Hitachi);电泳仪(Bio-Rad);凝胶成像系统(Bio-Rad);转膜设备(Bio-Rad);酶标仪(Tecan-M200pro);高速离心机(Heraeus)。

1.2 实验方法

1.2.1 RNA 的制备及 mRNA 表达测定 用 Trizol 试剂从经过处理的 C6 细胞株提取总体 RNA。cDNA 由 Transcriptor First Strand cDNA Synthesis kit(Roche)合成,然后利用 LC3、Beclin 1 和 Bcl2 基因特异性引物，序列如表 1 所示，用 FastStart Universal SYBR Green Master (Roche)，在 Mx3000P QPCR System 实时荧光定量 PCR 仪(Agilent technologies)上，对 LC3 、Beclin 1 和 Bcl2 的 cDNA 进行 PCR 扩增，数据用 MxPro QPCR 软件(Agilent)进行分析。各目标基因的 mRNA 表达量通过 GAPDH mRNA 表达量来进行标准化校正,且其表达量表示为相对于阳性对照细胞 C6 的相对表达量。对照组的 mRNA 表达设定为 1,每个样本测定三次,数据表示为均值± 标准差。

1.2.2 Western 检测 50 μL RIPA 蛋白裂解液裂解预先处理好的细胞，刮下细胞转移到 EP 管，冰上静置 30 min,13500 r/min,离心 15 min 后,收集上清液。参照 BCA 蛋白浓度测定试剂盒说明书,在样品孔和标准品孔中添加 BCA 工作液,37℃静置 30 min 后使用酶标仪对吸光度值进行测定 (波长 562 nm)。应用 ELISA Calc 回归 / 拟合计算程序绘制标准曲线,对蛋白浓度进行计算。把蛋白样品和蛋白标记加上电泳。转膜后的 PVDF 膜封闭成功后与一抗在 4 ℃条件下孵育过夜。把漂洗后的 PVDF 膜在二抗稀释液中浸没,摇床震荡 1 h 后漂洗进行化学发光反应。ECL 显影剂显影,用 BIO-RAD 凝胶成像系统进行检测。

1.2.3 MTT 选取对数期生长的 C6 细胞进行实验。在 96 孔板内种植密度为 5000 个 / 孔的细胞。对照组不做处理,超声组使用超声处理。HMME 组使用 10 μg/mL HMME 处理。对于 SDT 组,将细胞与 HMME 在避光预培养 2 h,然后在不同的时间点进行超声处理,超声参数设为:频率:1 MHz,强度:0.5 W/cm^2,作用时间:2 min。为了确定自噬和 SDT 处理对 C6 胶质瘤细胞活性的作用，分别使用自噬抑制剂 3-MA 和氯喹在培养 HMME 1 h 之前添加。SDT 处理后再培养 72 h,往各个孔中加 MTT,添加量为 10 μL,然后继续培养 4 h,培养结束后避光加二甲基亚砜,添加量为 200 μL,震荡时间为 10 min。设波长为 490 nm,使用酶标仪进行测定吸光度值。

1.2.4 透射电子显微镜 60 mm 培养皿培养 C6 细胞 24 h,C6 细胞进行 SDT 处理,12 h 后消化 C6 细胞再进行离心,3000 r/min,时间 20 min。去上清之后用 2.5 %戊二醛进行固定,时间为 10 min。PBS 洗净戊二醛,用 0.1 %锇酸固定。双蒸水冲洗,经过乙醇梯度进行脱水。将包埋于环氧树脂中过夜,切片。应用 Hitachi H-7650 电子显微镜(Hitachi,H-7650)进行观察图像。

1.3 统计学分析

采用 SPSS 19.0 软件分析数据，结果采用均值± 标准差表示,多组间比较采用单因素方差分析,用 Student t-test 检验两组间的差异,以 P<0.05 为差异具有统计学意义。

2 结果

2.1 SDT 对 C6 细胞增殖的影响

采用 MTT 法检测各个时间点细胞活力,如图 1 所示,与对照组相比,SDT 组细胞存活率从 0 h 至 6 h 逐渐下降，从 12 h 至 72 h 细胞存活率逐渐升高,HMME 组与对照组相比无统计学差异,然而在 6 h、12 h、24 h 与对照组相比,单独超声组也降低了细胞的存活率。

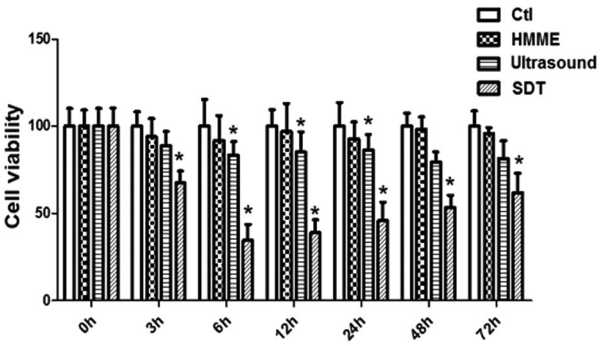

图 1 SDT 抑制 C6 胶质瘤细胞增殖

注:超声频率:1 MHz,强度 0.5 W/cm^2,照射时间 2 min。数据表示为平均值± SD,n=8。*P<0.05 相对 Ctl。Ctl:对照。

Fig.1 SDT inhibited proliferation of C6 glioma cells

Note: The ultrasonic frequency of 1MHz, intensity of 0.5 W/cm^2 and exposure time of 2 min. The data were represented as mean ± SD, n=8. *P<0.05 versus ctl. Ctl: control.

2.2 SDT 对 C6 胶质瘤细胞自噬的影响

如图 2 所示，透射电子显微镜分析结果显示 SDT 组自噬体数量较对照组明显增多。

2.3 SDT 对 C6 胶质瘤细胞中 LC3-II 表达水平的影响

如图 3 所示，与对照组相比,SDT 组 LC 3 蛋白明显升高(P<0.01)，超声组与对照组相比有统计学意义(P<0.05),HMME 组与对照组相比无统计学意义。如图 4 所示,与对照组相比,SDT 组 LC 3 mRNA 明显升高 (P<0.05)，超声组与 HMME 组和对照组相比无统计学意义。SDT 增加 C6 胶质瘤细胞中 LC 3 的表达。

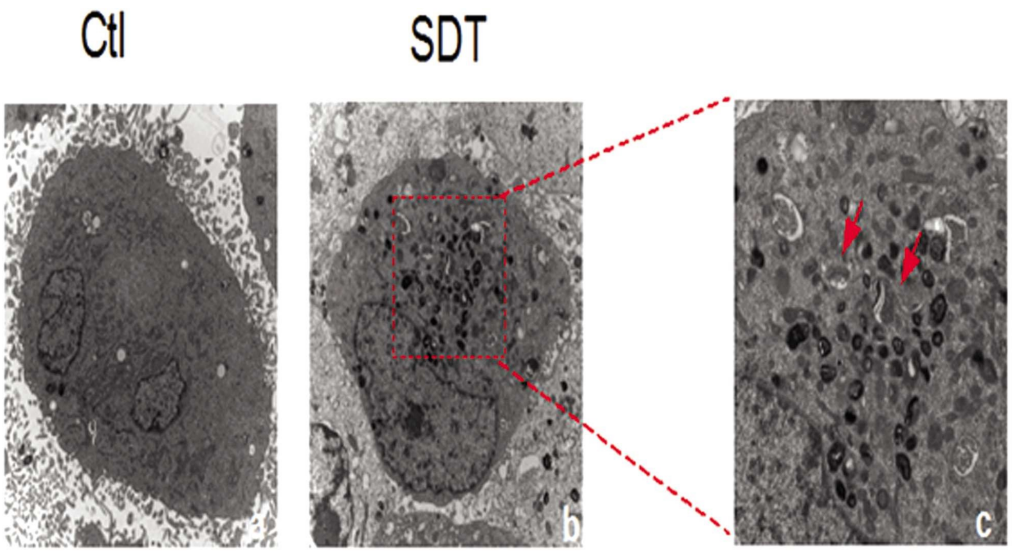

图 2 在 C6 胶质瘤细胞中 SDT 诱导自噬。

注:在 C6 胶质瘤细胞中自噬体的电镜分析。a,对照细胞(× 10,000)。b,SDT 处理的细胞,(× 10,000)。c,b 中放大的框,红色箭头表示自噬体(× 20,000)。 Ctl:对照。

Fig.2 SDT induced autophagy in C6 glioma cells

Note: Electron microscopic analysis of autophagosome in C6 glioma cells. a, control cells (× 10,000). b, SDT-treated cells, (× 10,000). c, enlarged box in b, the red arrows indicate autophagosome (× 20,000). Ctl: control.

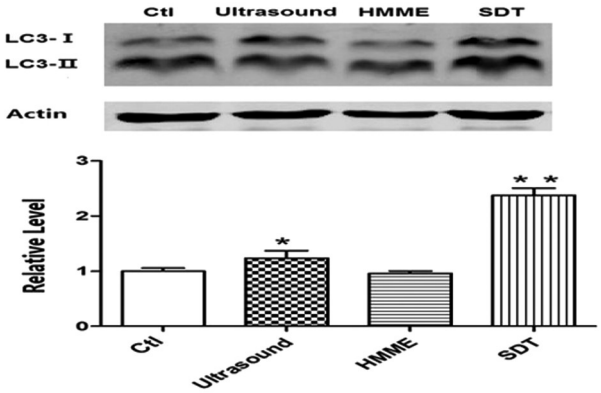

图 3 SDT 增加 LC3-II 蛋白表达水平。对照组,超声组,HMME 组和 SDT 组检测 LC3-II 水平。数据表示为平均值± SEM。*P<0.05 相对 ctl; **P<0.01 相对 Ctl。 Ctl:对照。

Fig.3 SDT increased LC3-II protein expression levels

The level of LC3-II was detected in control group, ultrasound group, HMME group and SDT group. The data were represented as mean ± SEM. *P<0.05 versus ctl; **P<0.01 versus ctl. Ctl: control.

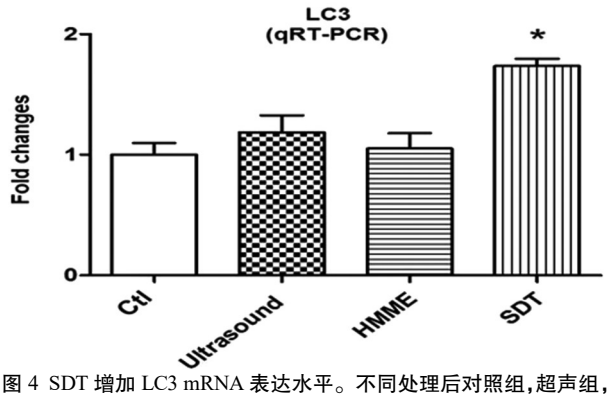

图 4 SDT 增加 LC3 mRNA 表达水平。不同处理后对照组,超声组,HMME 组和 SDT 组 LC3 的标准化定量分析。 数据表示为平均值± SEM。*P<0.05 相对 Ctl。 Ctl:对照

Fig.4 SDT increased LC3 mRNA expression levels

Normalized quantitative analysis of LC3 after different treatments in control group, ultrasound group, HMME group and SDT group. Data are expressed as the mean ± SEM. *P<0.05 versus ctl. Ctl: control.

2.4 SDT 对 C6 胶质瘤细胞中 Beclin1 表达水平的影响

如图 5 所示，与对照组相比,SDT 组 Beclin1 蛋白表达水平升高(P<0.05),超声组和 HMME 组与对照组相比无统计学意义。如图 6 所示,与对照组相比,SDT 组 Beclin1 mRNA 表达水平明显升高(P<0.01),超声组和 HMME 组与对照组相比无统计学意义。SDT 增加 C6 胶质瘤细胞中 Beclin1 的表达。

2.5 SDT 对 C6 胶质瘤细胞中 Bcl-2 表达水平的影响

如图 7 和图 8 所示，与对照组相比,SDT 组 Bcl-2 蛋白和 mRNA 表达水平降低(P<0.05),超声组与 HMME 组和对照组相比无统计学意义。SDT 降低 C6 胶质瘤细胞中 Bcl-2 的表达。

2.6 3-MA 或 CQ 联合 SDT 对 C6 胶质瘤细胞增殖的影响

如图 9 所示,SDT+3-MA 组 C6 胶质瘤细胞存活率低于 SDT 组,差异有统计学意义(P<0.05),SDT 组和对照组相比有统计学意义（P<0.01),3-MA 组和对照组相比有统计学意义(P<0.05)。如图 10 所示,SDT+CQ 组 C6 胶质瘤细胞存活率低于 SDT 组,差异有统计学意义(P<0.05),SDT 组和 CQ 组与对照组相比有统计学意义(P<0.05)。3-MA 或 CQ 联合 SDT 处理后增强了 C6 胶质瘤细胞的增殖抑制敏感性，说明自噬参与了 SDT 对 C6 胶质瘤细胞增殖的抑制作用。

3 讨论

胶质瘤属于常见的脑肿瘤,对化疗和放疗有抵抗性,具有较高复发率,单一治疗效果欠佳,迫切需要寻找新的联合治疗方式。SDT 作为治疗肿瘤的新疗法[16-18],与光动力疗法(Photo-

dynamic therapy ,PDT)相似。PDT 在胶质母细胞瘤中可以激活自噬，自噬激活作为促存活机制给予保护以抑制 PDT 诱导的坏死[19]。目前有 PDT,SDT 以及组合 PDT 和 SDT 的三种典型疗法[20]。SDT 依赖于超声和伴有较高的肿瘤组织亲和力药物的协同效应[21]。研究表明单独使用 10 μg/mL HMME 对 C6 胶质瘤细胞的细胞活性无影响,然而 HMME 与超声结合可导致 C6 胶质瘤细胞死亡[21]。因此,我们选择最终浓度为 10 μg / mL 的声敏剂 HMME。结果表明,超声联合 HMME 的处理能明显增加 C6 胶质瘤细胞在不同时间点的增殖抑制。

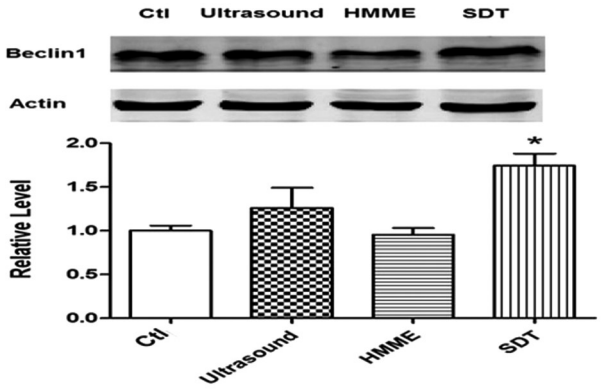

图 5 SDT 增加 Beclin 1 蛋白表达水平。对照组,超声组,HMME 组和 SDT 组检测 Beclin 1 水平。数据表示为平均值± SEM。*P<0.05 相对 Ctl。Ctl:对照。

Fig.5 SDT increased Beclin 1 protein expression levels

The level of Beclin 1 was detected in control group, ultrasound group, HMME group and SDT group. The data were represented as mean ± SEM. *P<0.05 versus ctl. Ctl: control.

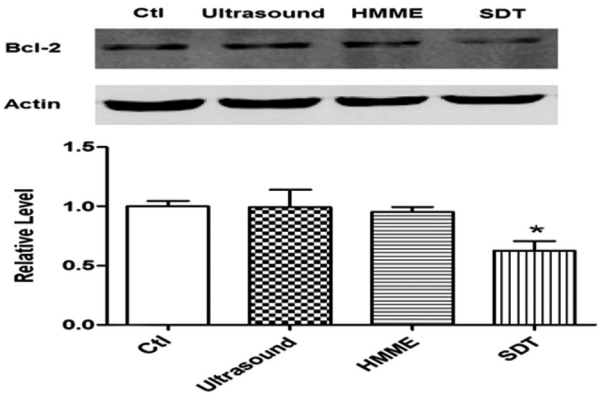

图 7 SDT 降低 Bcl-2 蛋白表达水平

注:对照组,超声组,HMME 组和 SDT 组中检测 Bcl-2 水平。数据表示为平均值± SEM。*P<0.05 相对 Ctl。Ctl:对照。

Fig.7 SDTdecreased Bcl-2 protein expression levels

Note: The level of Bcl-2 was detected in control group, ultrasound group, HMME group and SDT group. The upper panel is the representative band. The lower panel is the digital analysis. The data were represented as mean ± SEM. *P<0.05 versus ctl. Ctl: control.

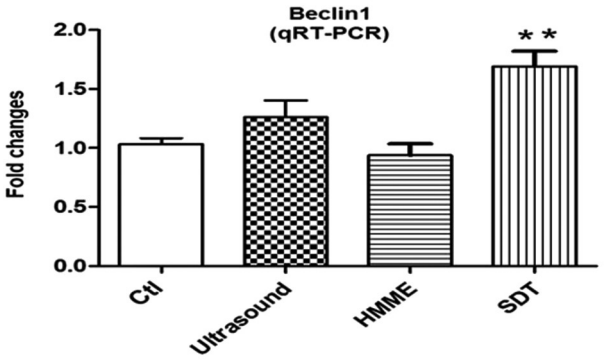

图 6 SDT 增加 Beclin1 mRNA 表达水平。用β-actin 标准化的 Beclin 1 定量分析不同处理的对照组,超声组,HMME 组和 SDT 组。数据表示为平均值± SEM。相比 *P<0.05 相对 ctl; **P<0.01 相对 ctl。Ctl:对照。

Fig.6 SDT increased Beclin1 mRNA expression levels

Quantitative analysis of Beclin 1 normalized with β-actin after different treatments in control group, ultrasound group, HMME group and SDT group. Data are expressed as the mean ± SEM. *P<0.05 versus ctl; **P<0.01 versus ctl. Ctl: control.

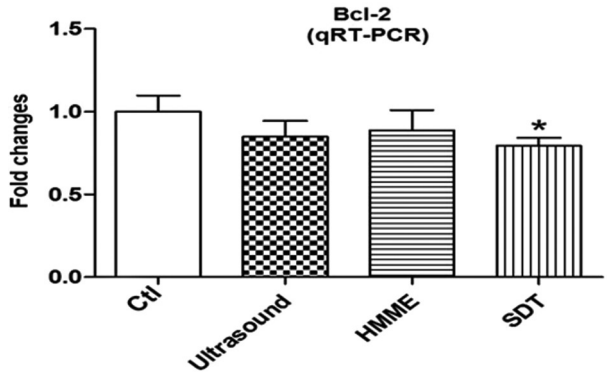

图 8 SDT 降低 Bcl2 mRNA 表达水平

注:β-actin 标准化的 Bcl2 定量分析不同处理后的对照组,超声组,HMME 组和 SDT 组。数据表示为平均值± SEM。*P<0.05 相对 ctl 。Ctl:对照。

Fig.8 SDT decreased Bcl-2 mRNA expression levels

Note: Quantitative analysis of Bcl2 normalized with β-actin after different treatments in control group, ultrasound group, HMME group and SDT group. Data are expressed as the mean ± SEM. *P<0.05 versus ctl; Ctl: control.

研究表明 HMME 介导 SDT 的机制主要集中在细胞凋亡和坏死以解释 SDT 诱导的细胞死亡[22-24]。因此,确定 SDT 的其它机制迫在眉睫，为胶质瘤患者临床应用 SDT 提供理论依据和新的潜在策略。研究表明 SDT 可以对细胞自噬产生影响[25],本研究也表明,在 C6 胶质瘤细胞中的 SDT 诱导自噬,具体体现在自噬体数目增加和 LC3-II 水平升高。

作为抑癌基因,Beclin1 在自噬和人类肿瘤之间的关系明确[26,27],Beclin1 阳性表达可能是胶质瘤患者的一个有用的预后因素[28]。在本研究中,SDT 组 Beclin 1 水平明显升高,这至少在某种程度上解释了 SDT 抑制 C6 胶质瘤细胞增殖的原因。更重要的是,自噬与肿瘤发生之间的信号通路存在许多重叠。原癌蛋白 Bcl-2 以介导肿瘤发生而众所周知[29],抑制 Bcl-2 的表达可能是胶质瘤未来治疗的一个方向[30],然而,它具有减少 Beclin1 复合体来抑制自噬的能力。本研究结果显示,SDT 组 Bcl-2 水平降低伴有 Beclin1 水平的升高，进一步证实 SDT 能诱导 C6 细胞自噬及降低 C6 胶质瘤细胞的增殖。3-MA 或 CQ

联合 SDT 处理后增强了 C6 胶质瘤细胞的增殖抑制敏感性，说明自噬参与了 SDT 对 C6 胶质瘤细胞增殖的抑制作用。

综上所述，本研究表明 SDT 可能通过诱导自噬抑制 C6 胶质瘤细胞增殖，为 HMME 介导的 SDT 临床应用提供了理论依据和新的潜在策略。

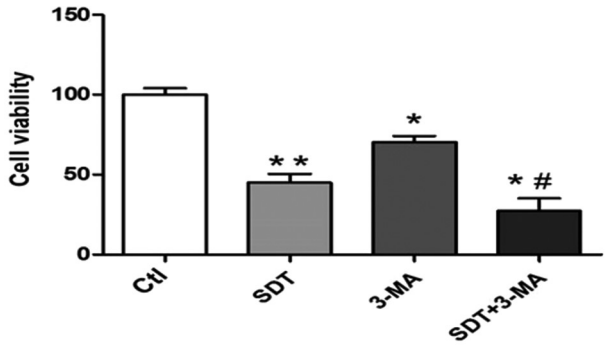

图 9 3-MA 对 C6 胶质瘤细胞增殖的影响。数据表示为平均值± SEM。 *P<0.05 相对 ctl;#P<0.05 相对 SDT。Ctl：对照; 3-MA:3- 甲基腺嘌呤。

Fig.9 Effect of 3-MA on proliferation of C6 glioma cells. Data are expressed as the mean ± SEM. *P<0.05 versus ctl; #P<0.05 versus SDT. Ctl: control; 3-MA: 3-methyladenine.

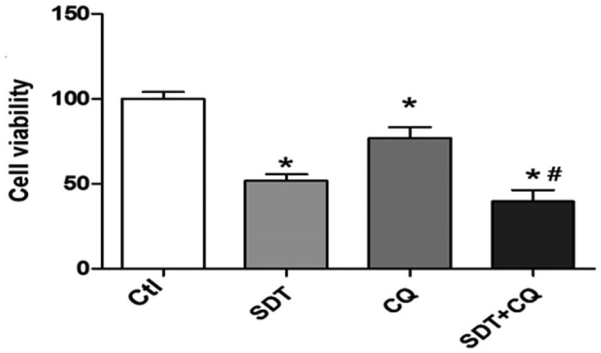

图 10 CQ 对 C6 胶质瘤细胞增殖的影响。数据表示为平均值± SEM。 *P<0.05 相对 ctl; #P<0.05 相对 SDT。Ctl：对照; CQ：氯喹。

Fig.10 Effect of CQ on proliferation of C6 glioma cells. Data are expressed as the mean ± SEM. *P<0.05 versus ctl; #P<0.05 versus SDT. Ctl: control; CQ: chloroquine.

参 考 文 献(References)

[1] Baradaran Rahimi V, Mousavi SH, Haghighi S, et al. Cytotoxicity and apoptogenic properties of the standardized extract of Portulaca oleracea on glioblastoma multiforme cancer cell line (U-87): a mechanistic study[J]. EXCLI J, 2019, 18: 165-186

[2] Molinaro AM, Taylor JW, Wiencke JK, et al. Genetic and molecular epidemiology of adult diffuse glioma [J]. Nat Rev Neurol, 2019, 15 (7): 405-417

[3] Chen L, Cong D, Li Y, et al. Combination of sonodynamic with temozolomide inhibits C6 glioma migration and promotes mitochondrial pathway apoptosis via suppressing NHE-1 expression [J]. Ultrason Sonochem, 2017, 39: 654-66

[4] Geng C, Zhang Y, Hidru TH, et al. Sonodynamic therapy: A potential treatment for atherosclerosis[J]. Life Sci, 2018, 207: 304-313

[5] Wan Q, Zou C, Hu D, et al. Imaging-guided focused ultrasound-induced thermal and sonodynamic effects of nanosonosensitizers for synergistic enhancement of glioblastoma therapy [J]. Biomater Sci, 2019, 7(7): 3007-3015

[6] Yoshida M, Kobayashi H,Terasaka S, et al.Sonodynamic Therapy for Malignant Glioma Using 220-kHz Transcranial Magnetic Resonance Imaging-Guided Focused Ultrasound and 5-Aminolevulinic acid [J]. Ultrasound Med Biol, 2019, 45(2): 526-538

[7] Pi Z, Huang Y, She Y. Sonodynamic Therapy on Intracranial Glioblastoma Xenografts Using Sinoporphyrin Sodium Delivered by Ultrasound with Microbubbles[J]. Ann Biomed Eng, 2019, 47(2): 549-562

[8] Wang MM, Feng YS, Yang SD, et al. The Relationship Between Autophagy and Brain Plasticity in Neurological Diseases [J]. Front Cell Neurosci, 2019, 13: 228

[9] McLoughlin F, Augustine RC, Marshall RS, et al. Maize multi-omics reveal roles for autophagic recycling in proteome remodelling and lipid turnover[J]. Nat Plants, 2018, 4(12): 1056-1070

[10] Gudipaty SA, Conner CM, Rosenblatt J, et al. Unconventional Ways to Live and Die: Cell Death and Survival in Development, Homeostasis, and Disease[J]. Annu Rev Cell Dev Biol, 2018, 34: 311-332

[11] Kaza N, Kohli L, Roth KA. Autophagy in brain tumors: a new target for therapeutic intervention[J]. Brain Pathol, 2012, 22(1): 89-98

[12] Boya P, Kroemer G.Inhibition of macroautophagy triggers apoptosis [J]. Mol Cell Biol, 2005, 25(3): 1025-1040

[13] Chaves CF, Mazzotti DR, Cendoroglo MS, et al. Genes related to maintenance of autophagy and successful aging[J]. Arq Neuropsiquiatr, 2018, 76(12): 831-839

[14] Sun Y, Wang H, Wang P, et al. Tumor targeting DVDMS-nanoliposomes for an enhanced sonodynamic therapy of gliomas[J]. Biomater Sci, 2019, 7(3): 985-994

[15] Li Q, Liu Q, Wang P, et al. The effects of Ce6-mediated sono-photodynamic therapy on cell migration, apoptosis and autophagy in mouse mammary 4T1 cell line[J]. Ultrasonics, 2014, 54(4): 981-989

[16] Teranishi R, Matsuda T, Yuba E, et al. Sonodynamic Therapeutic Effects of Sonosensitizers with Different Intracellular Distribution Delivered by Hollow Nanocapsules Exhibiting Cytosol Specific Release [J]. Macromol Biosci, 2019, 19(4): e1800365

[17] Zhang L, Yi H, Song J, et al. Mitochondria-Targeted and Ultrasound-Activated Nanodroplets for Enhanced Deep-Penetration Sonodynamic Cancer Therapy[J]. ACS Appl Mater Interfaces, 2019, 11(9): 9355-9366

[18] Huang J, Liu F, Han X, et al. Nanosonosensitizers for Highly Efficient Sonodynamic Cancer Theranostics [J]. Theranostics, 2018, 8 (22): 6178-6194

[19] Coupienne I, Bontems S, Rubio N, et al. NF-kappaB inhibition improves the sensitivity of human glioblastoma cells to 5-aminolevulinic acid-based photodynamic therapy [J]. Biochemical Pharma cology, 2011, 81(5): 606-1620

[20] Yang Y, Tu J, Yang D, et al. Photo- and Sono-Dynamic Therapy: A Review of Mechanisms and Considerations for Pharmacological Agents Used in Therapy Incorporating Light and Sound[J]. Curr Pharm Des, 2019, 25(4): 401-412

（下转第 3652 页）

[16] Alouini S, Mesnard L, Megier P, et al. Management of umbilical cord prolapse and neonatal outcomes [J]. J Gynecol Obstet Biol Reprod (Paris), 2010, 39(6): 471-477

[17] Flentje M, Schott M, Woltemate AL, et al. Interdisciplinary Simulation of Emergency Caesarean Section to Improve Subjective Competence[J]. Z Geburtshilfe Neonatol, 2017, 221(5): 226-234

[18] Hasegawa J, Sekizawa A, Ikeda T, et al. Clinical risk factors for poor neonatal outcomes in umbilical cord prolapse [J]. J Matern Fetal Neonatal Med, 2016, 29(10): 1652-1656

[19] Tashfeen K, Patel M, Hamdi IM, et al. Decision-to-Delivery Time Intervals in Emergency Caesarean Section Cases: Repeated cross-sectional study from Oman [J]. Sultan Qaboos Univ Med J, 2017, 17(1): e38-e42

[20] Ali Y, Khan MW, Mumtaz U, et al. Identification of factors influencing the rise of cesarean sections rates in Pakistan, using MCDM[J]. Int J Health Care Qual Assur, 2018, 31(8): 1058-1069

[21] Gibbons C, O'Herlihy C, Murphy JF. Umbilical cord prolapse--changing patterns and improved outcomes: a retrospective cohort study[J]. BJOG, 2014, 121(13): 1705-1708

[22] Kaymak O, Iskender C, Ibanoglu M, et al. Retrospective evaluation of risk factors and perinatal outcome of umbilical cord prolapse during labor[J]. Eur Rev Med Pharmacol Sci, 2015, 19(13): 2336-2339

[23] Hasegawa J, Ikeda T, Sekizawa A, et al. Obstetric risk factors for umbilical cord prolapse: a nationwide population-based study in Japan [J]. Arch Gynecol Obstet, 2016, 294(3): 467-472

[24] Kawakita T, Huang CC, Landy HJ. Risk Factors for Umbilical Cord Prolapse at the Time of Artificial Rupture of Membranes[J]. AJP Rep, 2018 ,8(2): e89-e94

[25] Hasegawa J, Sekizawa A, Ikeda T, et al. The use of balloons for uterine cervical ripening is associated with an increased risk of umbilical cordprolapse: population based questionnaire survey in Japan [J]. BMC Pregnancy Childbirth, 2015, 22; 15:4

[26] Portz S, Schmidt M, Weitz M. Lying. Down after Premature Rupture of the Membranes in Term Singleton Pregnancies: An Evidence-Based Recommendation? [J]. Z Geburtshilfe Neonatol, 2016, 220(5): 200-206

[27] Wasswa EW, Nakubulwa S, Mutyaba T. Fetal demise and associated factors following umbilical cord prolapse in Mulago hospital, Uganda: a retrospective study[J]. Reprod Health, 2014, 1,11(1): 12

[28] Rajakumar C, Garber A, Rao PM, et al. Umbilical Cord Prolapse in a Labouring Patient: A Multidisciplinary and Interprofessional Simulation Scenario[J]. Cureus, 2017, 9(9): e1692

[29] Copson S, Calvert K, Raman P, et al. The effect of a multidisciplinary obstetric emergency team training program, the In Time course, on diagnosis to delivery interval following umbilical cord prolapse - A retrospective cohort study [J]. Aust N Z J Obstet Gynaecol, 2017, 57(3): 327-333

[30] Siassakos D, Hasafa Z, Sibanda T, et al. Retrospective cohort study of diagnosis-delivery interval with umbilical cord prolapse: the effect of team training[J]. BJOG, 2009, 116(8): 1089-1096

[31] Calvert KL, McGurgan PM, Debenham EM, et al. Emergency obstetric simulation training: how do we know where we are going, if we don't know where we have been? [J]. Aust N Z J Obstet Gynaecol, 2013, 53(6): 509-516

（上接第 3620 页）

[21] Dai S, Wu C. Apoptotic effect of sonodynamic therapy mediated by hematoporphyrin monomethyl ether on C6 glioma cells in vitro [J]. Acta Neurochir (Wien), 2009, 151(12): 1655-1661

[22] Wang H, Yang Y, Sun X, et al. Sonodynamic therapy-induced foam cells apoptosis activates the phagocytic PPARγ-LXRα-ABCA1/ABCG1 pathway and promotes cholesterol efflux in advanced plaque [J]. Theranostics, 2018, 8(18): 4969-4984

[23] Sun X, Guo S, Wang W, et al. Potential involvement of the 18 kDa translocator protein and reactive oxygen species in apoptosis of THP-1 macrophages induced by sonodynamic therapy [J]. PLoS One, 2018, 13(5): e0196541

[24] Suehiro S, Ohnishi T, Yamashita D, et al. Enhancement of antitumor activity by using 5-ALA-mediated sonodynamic therapy to induce apoptosis in malignant gliomas: significance of high-intensity focused ultrasound on 5-ALA-SDT in a mouse glioma model[J]. J Neurosurg, 2018, 129(6): 1416-1428

[25] Li Q, Kang J, Xiong X, et al. Protoporphyrin IX-mediated sonodynamic therapy promotes autophagy in vascular smooth muscle cells [J]. Oncol Lett, 2017, 14(2): 2097-2102

[26] Zheng J, Shao Y, Jiang Y, et al. Tangeretin inhibits hepatocellu lar carcinoma proliferation and migration by promoting autophagy-related BECLIN1[J]. Cancer Manag Res, 2019, 11: 5231-5242

[27] Cao Y, Luo Y, Zou J, et al. Autophagy and its role in gastric cancer [J]. Clin Chim Acta, 2019, 489: 10-20

[28] Tamrakar S, Yashiro M, Kawashima T, et al. Clinicopathological Significance of Autophagy-related Proteins and its Association With Genetic Alterations in Gliomas [J]. Anticancer Res, 2019, 39 (3): 1233-1242

[29] Deng Y, Chen X, Huang C, et al. EZH2/Bcl-2 Coexpression Predicts Worse Survival in Diffuse Large B-cell Lymphomas and Demonstrates Poor Efficacy to Rituximab in Localized Lesions [J]. J Cancer, 2019, 10(9): 2006-2017

[30] Zhang X, Yu J, Zhao C, et al. MiR-181b-5p modulates chemosensitivity of glioma cells to temozolomide by targeting Bcl-2 [J]. Biomed Pharmacother, 2019, 109: 2192-2202