论著·“乳腺癌精准靶向与转化”重点号

分子伴侣自噬通过缓解G0/G1期阻滞维持细胞周期蛋白表达促进HER2阳性乳腺癌SK-BR-3细胞卡铂耐药

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［摘要］ **目的** 卡铂可通过诱导DNA损伤和细胞周期阻滞来抑制肿瘤细胞增殖,而细胞周期异常调控可能参与卡铂耐药,但具体调控机制尚待进一步阐明。本研究旨在探讨分子伴侣自噬(chaperone-mediated autophagy,CMA)对人表皮生长因子受体2(human epidermal growth factor receptor 2, HER2)阳性乳腺癌SK-BR-3细胞卡铂耐药的影响及其与细胞周期调控的关系。**方法** ①慢病毒感染构建CMA活性不同的SK-BR-3细胞株,通过荧光显微镜观察感染荧光,Western blot检测溶酶体相关膜蛋白2A(lysosome-associated membrane protein 2A,LAMP2A)表达,并结合LysoTracker Red免疫荧光评价CMA活性。②采用MTT法检测不同浓度卡铂处理后的细胞活力并确定半数抑制浓度(half-maximal inhibitory concentration, IC_{50});根据IC_{50}测定结果,以20 μmol/L卡铂连续处理各组细胞7 d,检测细胞增殖变化,并观察不同浓度卡铂处理后细胞密度及贴壁状态。③对高CMA活性组及其对照组进行RNA-seq测序,将差异表达基因(differentially expressed genes,DEGs)与细胞周期相关基因取交集,并进行GO和KEGG富集分析。④流式细胞术检测卡铂处理后各组细胞周期分布,Western blot检测CDK4、CyclinD1和CyclinE1的表达水平。**结果** ①成功构建CMA活性差异的SK-BR-3细胞模型。与shControl组相比,shLAMP2A组LAMP2A蛋白表达降低(P=0.000 3),LysoTracker Red荧光强度降低(P<0.000 1);与Control vector组相比,pCMV6-LAMP2A组LAMP2A蛋白表达升高(P<0.000 1),LysoTracker Red荧光强度升高(P=0.000 2)。②MTT结果显示,卡铂对SK-BR-3细胞的IC_{50}约为20 μmol/L;20 μmol/L卡铂连续处理7 d后,shLAMP2A组细胞增殖水平低于shControl组,pCMV6-LAMP2A组细胞增殖水平高于Control vector组(P<0.000 1);随着卡铂浓度升高,各组贴壁细胞数量总体减少,shLAMP2A组较shControl组减少更明显,而pCMV6-LAMP2A组较Control vector组则保留较多贴壁细胞。③RNA-seq及富集分析显示,高CMA活性相关差异基因主要富集于有丝分裂细胞周期转变、CDK复合物、DNA解旋酶活性及细胞周期相关通路。④流式细胞术和Western blot结果进一步显示,与shControl组相比,shLAMP2A组G0/G1期细胞比例增加(P=0.000 4),S期细胞比例降低(P=0.024 3);与Control vector组相比,pCMV6-LAMP2A组G0/G1期细胞比例降低(P<0.000 1),S期细胞比例升高(P=0.000 3)。shLAMP2A组CDK4、CyclinD1和CyclinE1表达较shControl组降低,而pCMV6-LAMP2A组较Control vector组升高(P<0.000 1)。**结论** CMA活性增强可减弱卡铂对SK-BR-3细胞的增殖抑制作用,提高细胞在卡铂压力下的存活和增殖能力;其机制可能与缓解G0/G1期阻滞、维持CDK4、CyclinD1和CyclinE1表达及促进G1/S期转换有关。CMA介导的细胞周期调控可能参与SK-BR-3细胞卡铂耐药。

［关键词］ 分子伴侣自噬;HER2阳性乳腺癌;卡铂;耐药;细胞周期

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Chaperone-mediated autophagy promotes carboplatin resistance in HER2-positive breast cancer SK-BR-3 cells by alleviating G0/G1 arrest and maintaining cell cycle protein expression

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[**Abstract**] **Objective** Carboplatin inhibits tumor cell proliferation by inducing DNA damage and cell cycle arrest. Whereas abnormal regulation of the cell cycle may be involved in carboplatin resistance, the underlying regulatory mechanisms remain to be further elucidated. This study aims to investigate the effect of chaperone-mediated autophagy (CMA) on carboplatin resistance in HER2-positive breast cancer SK-BR-3 cells and its relationship with cell cycle regulation. **Methods** ① SK-BR-3 cell lines with different levels of CMA activity were established by lentiviral transduction. Transduction-associated fluorescence was observed by fluorescence microscopy, LAMP2A protein expression was detected by Western blotting, and CMA activity was evaluated in combination with LysoTracker immunofluorescence. ② Cell viability after treatment with different concentrations of carboplatin was assessed using MTT assay to determine the half-maximal inhibitory concentration (IC_{50}). Based on the IC_{50} value, the cells in each group were continuously treated with 20 μmol/L carboplatin for 7 d to evaluate changes in cell proliferation, and cell density and attachment status were observed after treatment with different concentrations of carboplatin. ③ RNA sequencing (RNA-seq) was performed on the high-CMA-activity group and its corresponding control group. Differentially expressed genes (DEGs) were intersected with cell cycle-related genes, followed by GO and KEGG enrichment analyses. ④ Cell cycle distribution after carboplatin treatment was analyzed by flow cytometry, and the expression levels of CDK4, CyclinD1, and CyclinE1 were detected by Western blotting. **Results** ① SK-BR-3 cell models with different levels of CMA activity were successfully established. Compared with the shControl group, the shLAMP2A group showed significantly decreased LAMP2A protein expression (P=0.000 3) and decreased LysoTracker Red fluorescence intensity (P<0.000 1). Compared with the Control vector group, the pCMV6-LAMP2A group showed increased LAMP2A protein expression (P<0.000 1) and enhanced LysoTracker Red fluorescence intensity (P=0.000 2). ② MTT assay indicated that the IC_{50} value of carboplatin against SK-BR-3 cells was approximately 20 μmol/L. After continuous treatment with 20 μmol/L carboplatin for 7 d, cell proliferation was lower in the shLAMP2A group than the shControl group, whereas it was higher in the pCMV6-LAMP2A group than in the Control vector group (P<0.000 1). With the increasing of the carboplatin concentration, the number of adherent cells was generally decreased in all groups; this decrease was more pronounced in the shLAMP2A group than in the shControl group, whereas more adherent cells were retained in the pCMV6-LAMP2A group. ③ RNA-seq and enrichment analyses showed that DEGs associated with high CMA activity were mainly enriched in mitotic cell cycle transition, CDK complexes, DNA helicase activity, and cell cycle-related pathways. ④ Flow cytometry and Western blotting further revealed that, compared with the shControl group, the proportion of cells in G0/G1 phase was increased (P=0.000 4) and that in S phase was decreased (P=0.024 3) in the shLAMP2A group. Compared with the Control vector group, the pCMV6-LAMP2A group showed a decreased proportion of cells in G0/G1 phase (P<0.000 1) and an increased proportion of cells in S phase (P=0.000 3). The expression levels of CDK4, CyclinD1, and CyclinE1 were notably lower in the shLAMP2A group than the shControl group, whereas that in the pCMV6-LAMP2A group was higher than that in the Control vector group (P<0.000 1). **Conclusion** Enhanced CMA activity attenuates the inhibitory effect of carboplatin on SK-BR-3 cell proliferation and improves cell survival and proliferative capacity under carboplatin stress. The mechanism may be related to enhanced CMA activity alleviating G0/G1 phase arrest, maintaining CDK4, CyclinD1, and CyclinE1 expression, and promoting G1/S phase transition. CMA-mediated regulation of cell cycle may be involved in carboplatin resistance in SK-BR-3 cells.

[**Key words**] chaperone-mediated autophagy; HER2-positive breast cancer; carboplatin; drug resistance; cell cycle

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乳腺癌是女性最常见的恶性肿瘤之一,数据显示女性乳腺癌仍居女性恶性肿瘤发病首位[1]。乳腺癌具有显著的分子异质性,不同的分子分型决定了不同的系统治疗策略、药物反应和耐药机制[2]。SK-BR-3细胞来源于人表皮生长因子受体2(human epidermal growth factor receptor 2,HER2)高表达乳腺癌,是研究HER2阳性乳腺癌细胞生物学行为、药物反应及耐药机制的常用体外模型。尽管HER2靶向治疗显著改善了患者的治疗结局,但部分患者仍会发生原发或获得性耐药[3];同时,含铂方案在复发转移性及特定分子亚型乳腺癌中的应用仍受到关注。卡铂可通过形成DNA加合物和链间交联,阻碍DNA复制及激活DNA损伤反应诱导细胞周期停滞[4-5],若肿瘤细胞能够维持DNA损伤修复能力、逃逸检查点控制或重新启动细胞周期,则可能出现对铂类药物的耐受[6]。

分子伴侣自噬(chaperone-mediated autophagy,CMA)是一种选择性溶酶体蛋白降解途径,含KFERQ样基序的底物蛋白首先被热激蛋白(heat shock cognate protein 70,HSC70)识别并递送至溶酶体膜,随后与膜上的溶酶体相关膜蛋白2A(lysosomal associated membrane protein 2A,LAMP2A)结合并转运入溶酶体腔内降解[7-8]。LAMP2A被认为是CMA活性的关键限速分子,其表达和多聚化状态直接影响底物进入溶酶体的效率。近年来研究表明,CMA并非单纯的蛋白质质量控制系统,而是参与肿瘤细胞代谢重编程、氧化应激适应、转移侵袭以及药物耐受的重要调控网络[9-11]。在乳腺癌中,LAMP2A表达异常与肿瘤细胞存活和治疗抵抗相关;CMA还可通过调控糖酵解[12]、上皮间质转化[13-14]及转录因子稳定性等过程促进乳腺癌进展。

细胞周期调控是化疗耐药研究中的核心环节之一。CDK4/CyclinD1复合物驱动视网膜母细胞瘤(retinoblastoma,RB)蛋白磷酸化和转录因子(E2F transcription factor,E2F)释放,CyclinE1/CDK2进一步促进G1/S期转换及DNA复制启动,是细胞越过G1限制点的重要分子基础[15-16]。上述细胞周期调控通路异常激活可削弱DNA损伤诱导的细胞周期阻滞,使肿瘤细胞在药物压力下继续增殖。在HER2阳性乳腺癌中,CDK4/6相关通路还被认为与抗HER2治疗耐受及维持肿瘤细胞增殖有关[17-18]。既往研究已提示CMA可通过选择性降解底物蛋白、调节代谢与应激反应影响细胞增殖和周期进程[11-19]。然而,CMA是否参与HER2阳性乳腺癌细胞的G1/S期转换及卡铂耐受尚不明确。因此,本研究构建LAMP2A稳定敲低和过表达的SK-BR-3细胞株,结合MTT、RNA-seq、流式细胞术和Western blot,探讨CMA与卡铂耐受及细胞周期调控的关系。

1 材料与方法

1.1 试剂与材料

1.1.1 实验细胞 人HER2阳性乳腺癌细胞株SK-BR-3购自中国科学院上海细胞库。

1.1.2 主要试剂 卡铂(carboplatin)购自MedChemExpress(MCE)公司,溶于H_2O配制成储备液,避光保存,使用前以培养基稀释至实验浓度;高糖DMEM、青霉素-链霉素双抗、胰酶消化液、胎牛血清、LysoTracker™ Red DND-99购自赛默飞世尔科技公司;MTT试剂盒、抗荧光淬灭封片剂、Hoechst 33342、DAPI、Western及细胞裂解液和嘌呤霉素均购自碧云天生物技术有限公司;兔抗LAMP2A重组抗体购自英国Abcam公司;兔抗CDK4、CyclinD1和CyclinE1多克隆抗体、兔抗β-actin和Tubulin单克隆抗体以及HRP-conjugated Goat anti-Rabbit IgG(H+L)购自武汉三鹰生物技术有限公司;ECL试剂盒购自Advansta公司;二甲基亚砜(DMSO)购自北京索莱宝科技有限公司。

1.2 实验方法

1.2.1 慢病毒感染 慢病毒购自上海吉凯基因。取对数生长期SK-BR-3细胞,以5×10^5个/孔接种于6孔板中,培养24 h至融合度约50%~60%。分别采用LAMP2A敲低慢病毒、敲低空载病毒、LAMP2A过表达慢病毒及过表达空载病毒进行感染,对应记为shLAMP2A组、shControl组、pCMV6-LAMP2A组和Control vector组。感染12 h后更换新鲜完全培养基。96 h后加入浓度为1 μg/mL的嘌呤霉素,直至获得稳定细胞株,并通过Western blot验证LAMP2A表达。

1.2.2 Western blot检测蛋白的表达水平 提取细胞总蛋白,BCA试剂盒测蛋白浓度,调整蛋白上样量后,进行SDS-PAGE电泳,电泳完成后将蛋白转移到PVDF膜上,用5%脱脂奶粉溶液室温封闭2 h,TBST洗膜后于4 ℃孵育一抗过夜。分别按1∶5 000稀释比孵育CDK4、CyclinD1、CyclinE1、LAMP2A、β-actin、Tubulin等抗体。次日,按1∶5 000稀释HRP-conjugated Goat anti-Rabbit IgG(H+L)孵育二抗,TBST洗涤,在Tanon 4600成像系统中使用ECL底物通过增强化学发光显现目标蛋白质条带。

1.2.3 LysoTracker Red荧光染色 细胞融合度达60%~80%时,用PBS洗涤。以无血清培养基将LysoTracker Red DND-99稀释至75 nmol/L,加入细胞后于37 ℃、5% CO_2条件下避光孵育20 min。PBS洗涤后,加入Hoechst 33342工作液,使终浓度为5 μg/mL,继续避光孵育5 min。孵育完成后,吸弃染核试剂工作液,PBS洗涤。将培养板置于荧光显微镜下,根据LysoTracker Red(激发577 nm/发射590 nm)和Hoechst 33342(激发350 nm/发射460 nm)的激发及发射波长设置成像通道,调节焦距和曝光时间等参数,随机选取3~5个视野采集图像。

1.2.4 MTT法测定卡铂半数抑制浓度(half-maximal inhibitory concentration, IC_{50}) 细胞经0.25%胰酶消化后制成单细胞悬液,计数并调整细胞浓度。按每孔5 000个细胞接种于96孔培养板中,每孔加入100 μL细胞悬液,于37 ℃、5% CO_2及饱和湿度条件下培养24 h。待细胞完全贴壁后,弃去原培养液,分别加入含0、2.5、5、10、20、40、80、150和200 μmol/L卡铂的完全培养基,每孔总体积为100 μL,每个浓度设置6个复孔,其中0 μmol/L卡铂组作为对照,另设无细胞空白孔。各组细胞继续培养48 h。卡铂处理结束后,向各孔加入MTT工作液,于37 ℃条件下避光孵育3 h。孵育结束后弃去液体,加入100 μL DMSO,置于微孔板振荡器上振荡,使甲瓒结晶充分溶解。采用酶标仪在490 nm波长处测定各孔吸光度*OD*(490)值。

1.2.5 MTT生长曲线实验 根据IC_{50}测定结果,选择20 μmol/L作为后续实验浓度。将各组细胞制成单细胞悬液,计数后按4×10^3个/孔接种于96孔板,每孔加入100 μL完全培养基。细胞培养24 h后,更换为含20 μmol/L卡铂的完全培养基继续培养。于卡铂处理后第1至7天分别检测相应独立复孔,每个时间点设6个复孔。培养期间根据细胞生长状态更换培养液,换液后维持卡铂终浓度为20 μmol/L。

1.2.6 卡铂处理后细胞密度及贴壁状态观察 各组细胞按8×10^4个/孔接种于6孔培养板中,每孔加入2 mL完全培养基,于37 ℃、5% CO_2条件下培养24 h。待细胞贴壁后,弃去原培养液,分别加入含0、5、20和40 μmol/L卡铂的完全培养基,继续处理48 h。处理结束后,在相同放大倍数和成像参数下观察各组细胞密度以及贴壁状态等变化,并采集代表性图像。

1.2.7 RNA-seq及生物信息学分析 提取Control vector组和pCMV6-LAMP2A组细胞总RNA,每组设置3个生物学重复。质检合格后进行转录组测序。以差异表达倍数和统计学显著性筛选差异表达基因(differentially expressed genes, DEGs),并与细胞周期相关基因集取交集。采用GO数据库从生物过程(biological process, BP)、细胞组分(cellular component, CC)和分子功能(molecular function, MF)3个层面进行富集分析,同时采用KEGG数据库分析交集基因可能参与的信号通路。

1.2.8 流式细胞术检测细胞周期分布 各组细胞经20 μmol/L卡铂处理48 h后收集,将原培养基收集于离心管,加入不含EDTA的0.25%胰酶消化液消化细胞,并用原培养基终止消化;离心5 min(1 000 r/min)后弃上清,用PBS清洗2次。将细胞重悬于100 μL PBS中,缓慢加入500 μL预冷75%乙醇,边加边振荡以避免细胞聚集,并于4 ℃固定。检测前弃去乙醇,用PBS洗涤后加入PI/RNase染色液,避光孵育后采用流式细胞仪检测细胞周期分布。

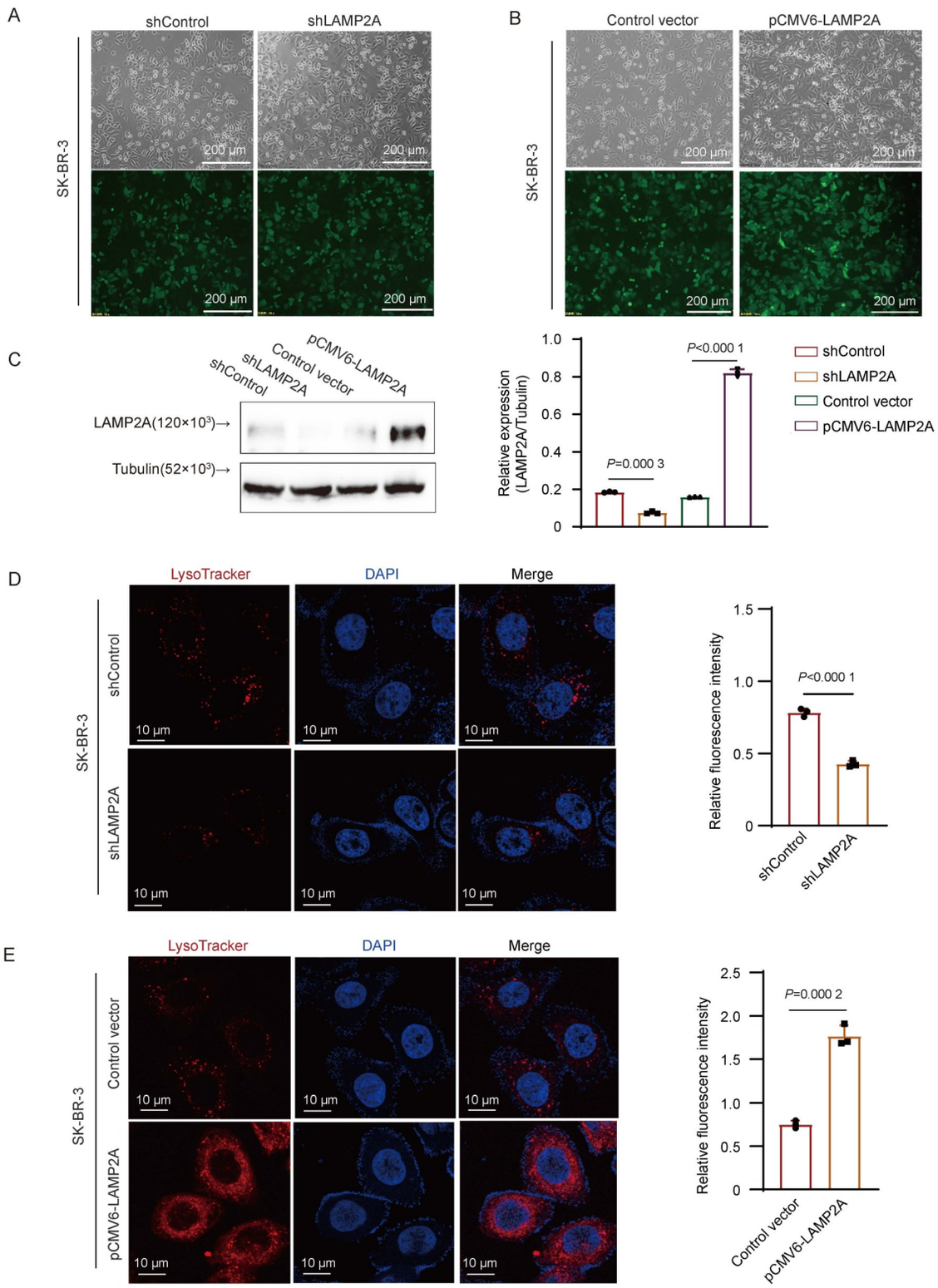
1.3 统计学分析

采用GraphPad Prism 8.4.2软件处理数据。使用ImageJ 1.8.0软件分析CDK4、CyclinD1、CyclinE1、LAMP2A的表达定量。各实验独立重复3次。经正态性检验后,符合正态分布的定量资料以$\bar{x}\pm s$表示;2组间比较采用t检验,多时间点或多因素数据根据实验设计采用双因素方差分析或在各时间点进行2组比较。$P<0.05$表示差异有统计学意义。

2 结果

2.1 构建CMA活性高低不同的SK-BR-3细胞株

通过荧光显微镜拍照观察,各组细胞均可见明显的荧光(图1A、B)。Western blot显示,shLAMP2A组中LAMP2A蛋白的表达水平较shControl组明显降低;而pCMV6-LAMP2A组,该蛋白的表达较Control vector组显著升高(图1C)。LysoTracker Red染色显示,与shControl组相比,shLAMP2A组荧光强度降低(图1D);与Control vector组相比,pCMV6-LAMP2A组荧光强度升高(图1E)。以上结果表明CMA活性高低不同的SK-BR-3细胞株构建成功。



A: Representative phase-contrast and fluorescence images of shControl and shLAMP2A cells following lentiviral transduction; B: Representative phase-contrast and fluorescence images of Control vector and pCMV6-LAMP2A cells following lentiviral transduction; C: Western blot and densitometric analyses of LAMP2A expression(n=3, $\bar{x}\pm s$); D: Representative LysoTracker Red fluorescence images and quantification in shControl and shLAMP2A cells(n=3, $\bar{x}\pm s$); E: Representative LysoTracker Red fluorescence images and quantification in Control vector and pCMV6-LAMP2A cells(n=3, $\bar{x}\pm s$)

图1 CMA活性不同的SK-BR-3稳定细胞株的构建与验证

Figure 1 Establishment and validation of stable SK-BR-3 cell lines with different levels of CMA activity

2.2 CMA活性改变影响卡铂处理下SK-BR-3细胞的增殖

结果显示,SK-BR-3细胞活力随药物浓度升高而逐渐下降,剂量-反应曲线显示卡铂对SK-BR-3细胞具有明确的增殖抑制作用(图2A)。根据IC_{50}测定结果,选择20 μmol/L作为后续实验作用浓度,以便进一步评价CMA调控后细胞对卡铂的敏感性差异。在20 μmol/L卡铂连续作用7 d条件下,shLAMP2A组细胞增殖速度低于shControl组(图2B);相反,pCMV6-LAMP2A组细胞增殖能力高于Control vector组(图2C)。上述结果表明,卡铂可有效抑制SK-BR-3细胞增殖,而CMA活性改变能够影响细胞对卡铂的增殖反应;低CMA活性增强卡铂抑制作用,高CMA活性则减弱卡铂抑制作用,提示CMA活性增强可能与SK-BR-3细胞卡铂耐受有关。

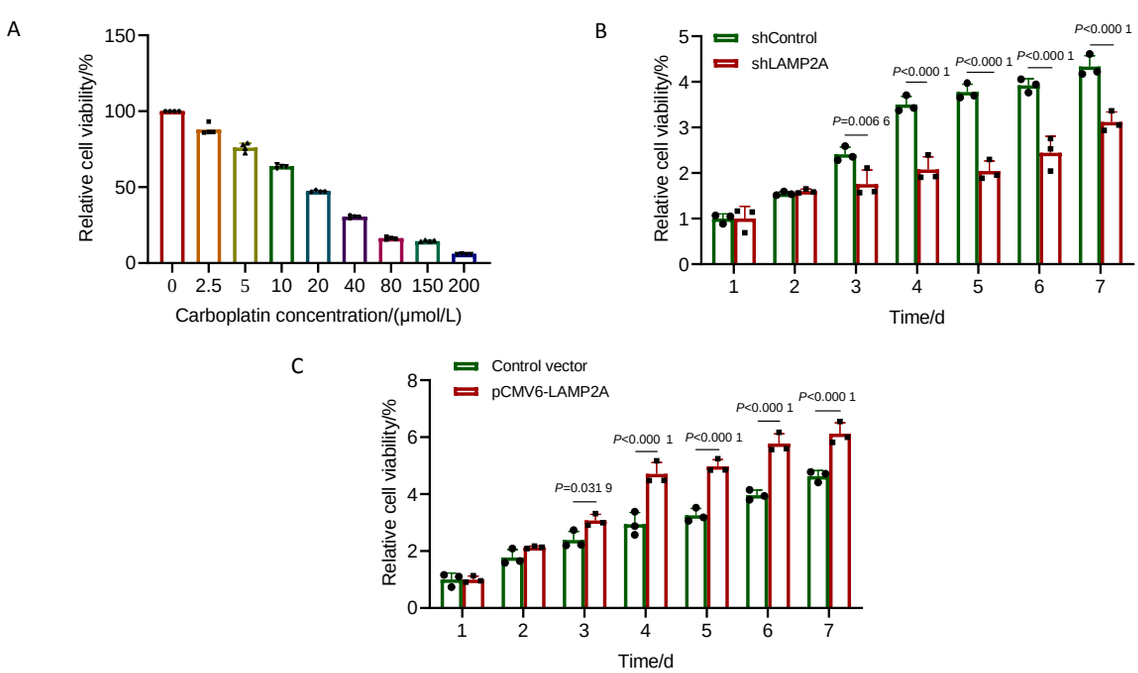
显微镜观察显示,随着卡铂浓度升高,各组视野内贴壁细胞数量总体呈减少趋势。与shControl相比,shLAMP2A组在20 μmol/L和40 μmol/L卡铂处理后贴壁细胞数量较少;与Control vector组相比,pCMV6-LAMP2A组在相同浓度卡铂处理后保留了较多贴壁细胞(图3A、B)。结合MTT结果,提示LAMP2A表达改变可能影响SK-BR-3细胞在卡铂处理条件下的增殖状态。

2.3 CMA活性相关基因主要富集于细胞周期调控过程

GO富集分析显示,交集基因在BP层面主要涉及蛋白稳定性调控、有丝分裂、细胞周期进程、DNA复制和检查点调控等过程(图4A);在CC层面主要富集于纺锤体、染色体、复制相关结构及细胞周期调控复合体(图4B);在MF层面则与蛋白结合、核酸结合、转录调控及细胞周期蛋白相关调控功能有关(图4C)。KEGG富集分析显示,交集基因涉及铂类药物耐药、凋亡以及多种肿瘤相关通路(图4D)。上述结果提示,细胞周期调控可能参与CMA介导的卡铂耐受。

2.4 CMA通过调控细胞周期影响SK-BR-3细胞卡铂耐药

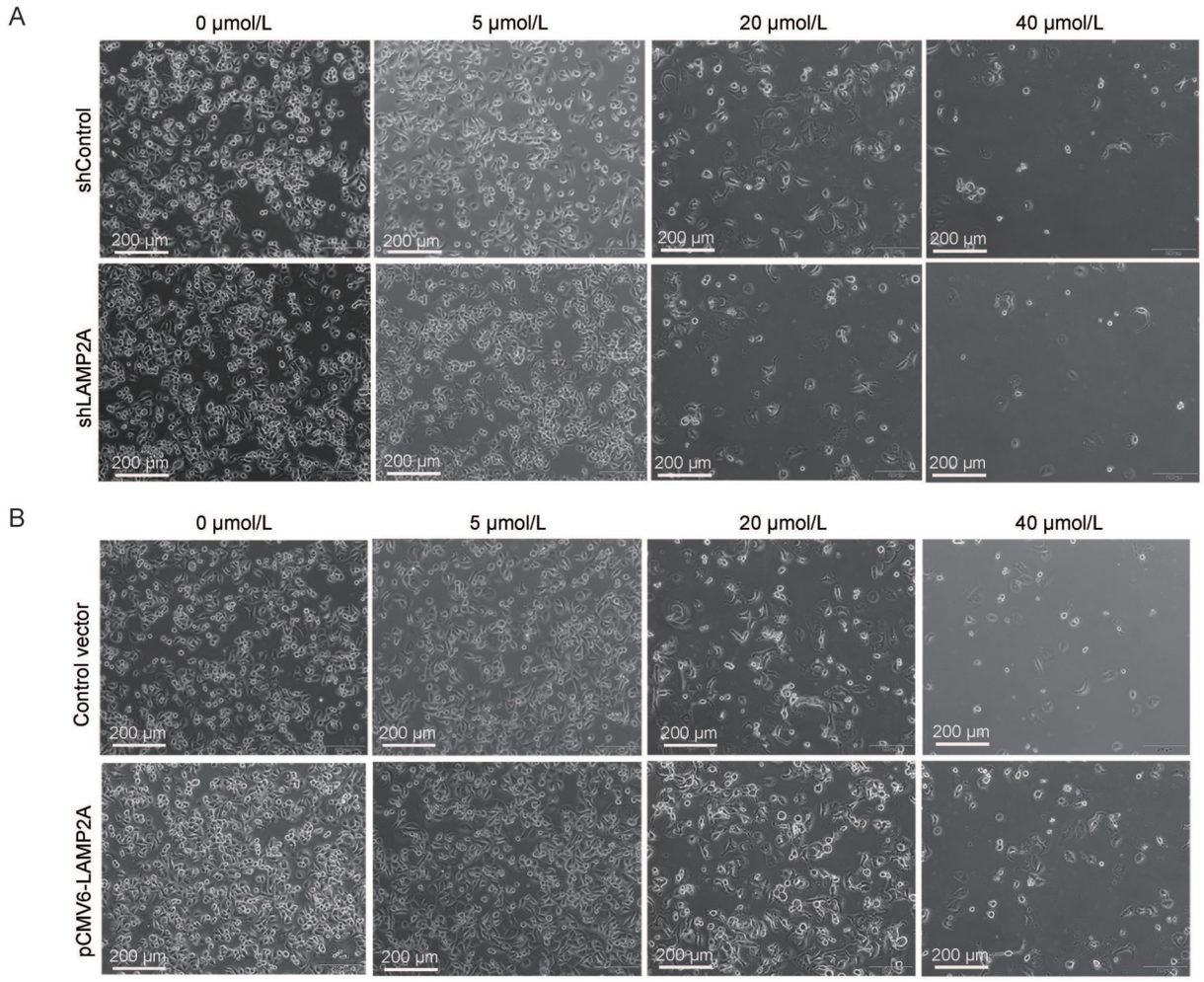
流式细胞术结果显示,与shControl组相比,shLAMP2A组的G0/G1期细胞比例显著增加,S期细胞比例显著减少,表明G0/G1期阻滞加剧(图5A);pCMV6-LAMP2A组G0/G1期细胞比例较Control vector组降低,进入S期的细胞比例则升高,提示G0/G1期阻滞减轻(图5B)。



A: MTT assay showing SK-BR-3 cell viability after treatment with 0 to 200 μmol/L carboplatin for 48 h and determination of the IC_{50} value; B: Proliferation of shControl and shLAMP2A SK-BR-3 cells exposed to 20 μmol/L carboplatin for 1 to 7 d; C: Proliferation of Control vector and pCMV6-LAMP2A SK-BR-3 cells exposed to 20 μmol/L carboplatin for 1 to 7 d

图2 卡铂对SK-BR-3细胞活力及CMA活性改变后细胞增殖的影响 (n=3, $\bar{x}\pm s$)

Figure 2 Effects of carboplatin on SK-BR-3 cell viability and cell proliferation after alteration of CMA activity (n=3, $\bar{x}\pm s$)



A: Representative phase-contrast images of shControl and shLAMP2A SK-BR-3 cells after treatment with 0, 5, 20, and 40 μmol/L carboplatin for 48 h; B: Representative phase-contrast images of Control vector and pCMV6-LAMP2A SK-BR-3 cells after treatment with 0, 5, 20, and 40 μmol/L carboplatin for 48 h

图3 不同CMA活性SK-BR-3细胞经不同浓度卡铂处理后的细胞密度及贴壁状态

Figure 3 Cell density and attachment of SK-BR-3 cells with different levels of CMA activity after treatment with various concentrations of carboplatin

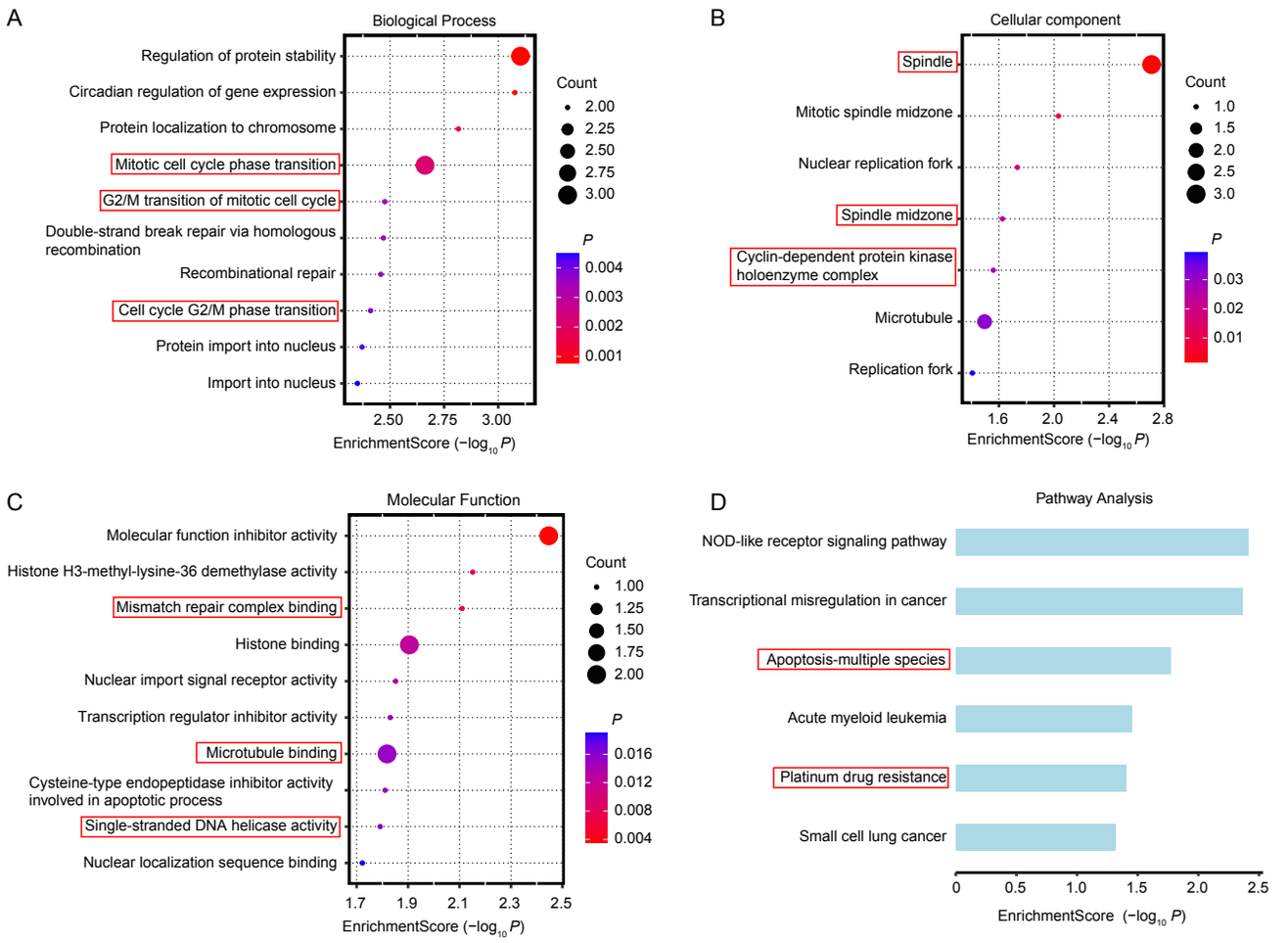
Western blot结果进一步显示,卡铂作用后,shLAMP2A组细胞周期相关蛋白CDK4、CyclinD1和CyclinE1表达较shControl组降低(图5C);而pCMV6-LAMP2A组蛋白表达较Control vector组升高(图5D)。CDK4、CyclinD1和CyclinE1共同参与G1/S期转换,上述结果提示,在卡铂处理条件下,LAMP2A表达改变与G1/S期相关蛋白表达及细胞周期分布变化有关。

3 讨论

铂类药物耐受是限制乳腺癌化疗获益的重要因素。卡铂通过形成DNA加合物、阻碍DNA复制和激活细胞周期检查点抑制肿瘤细胞增殖,而肿瘤细胞对DNA损伤修复、蛋白质稳态及细胞周期进程的重塑可削弱药物效应[4-6, 10]。本研究提示,CMA活性增强可能通过维持细胞周期进程、减轻G0/G1期阻滞而促进SK-BR-3细胞卡铂耐受。

3.1 CMA活性增强促进SK-BR-3细胞卡铂耐药

乳腺癌的发生发展、复发及治疗反应受到多种肿瘤细胞内在及微环境因素影响[20-21],其中DNA损伤反应的差异可影响肿瘤细胞对卡铂的敏感性及耐药表型[22]。CMA可通过选择性降解受损或功能异常蛋白,帮助细胞维持蛋白质稳态并适应氧化、代谢及药物压力[7-11, 23]。已有研究发现,使用药物抑制CMA可能具有一定的抗肿瘤作用[24]。细胞蛋白质稳态的维持依



A: GO enrichment analysis of biological process (BP); B: GO enrichment analysis of cellular component (CC); C: GO enrichment analysis of molecular function (MF); D: KEGG pathway enrichment analysis. The analyzed genes were obtained by intersecting RNA-seq-derived differentially expressed genes between pCMV6-LAMP2A and Control vector SK-BR-3 cells with cell cycle-related genes

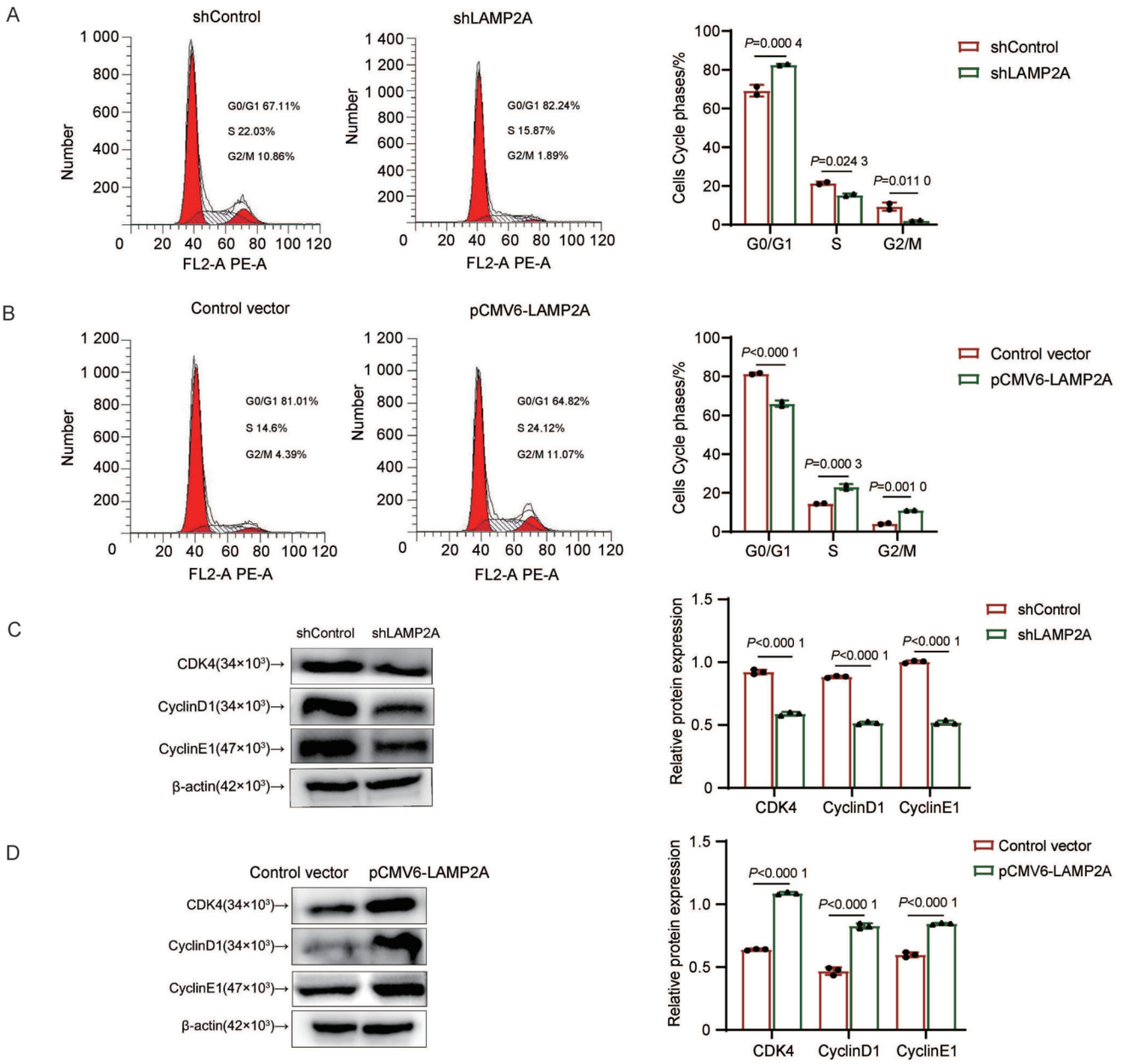
图4 高CMA活性相关差异基因与细胞周期相关基因的交集基因主要富集于细胞周期调控过程

Figure 4 High-CMA-activity-associated differentially expressed genes overlapping with cell cycle-related genes are mainly enriched in cell cycle regulatory processes

赖分子伴侣、蛋白酶体及自噬等多种质量控制途径[25];在乳腺癌中,CMA介导的特定底物降解还可参与肿瘤进展和转移[26]。在多种肿瘤中,LAMP2A上调与肿瘤细胞生存、增殖和化疗耐受增强有关;近期研究亦显示,LAMP2A可增强结直肠癌细胞对顺铂的耐受,而敲低LAMP2A可提高细胞对顺铂的敏感性[27]。本研究中,敲低LAMP2A增强了卡铂对细胞增殖的抑制作用,而过表达LAMP2A呈相反效应,提示CMA可能参与调控SK-BR-3细胞的卡铂敏感性。结合CMA在蛋白质质量控制、糖酵解和应激适应中的作用[9-13],推测增强CMA可能通过维持细胞稳态和增殖能力,降低卡铂对SK-BR-3细胞的抑制效应,从而降低细胞对卡铂的敏感性。

3.2 高CMA活性相关差异基因富集提示细胞周期参与卡铂耐药

卡铂诱导DNA损伤和干扰DNA复制过程,并通过检查点激活造成细胞周期停滞;逃逸检查点或重新启动细胞周期可使肿瘤细胞在药物作用下继续增殖,从而促进耐药[4-6]。LAMP2A过表达相关DEGs与细胞周期基因的交集主要富集于有丝分裂、CDK复合物、DNA复制及检查点调控。上述结果与高CMA活性组在卡铂作用下仍保持较强增殖能力的表型一致,提示细胞周期调控可能是连接CMA活性增强与卡铂耐受的关键环节。细胞越过G1限制点并进入S期需要多种周期蛋白和CDK协同作用[15-16],因此转录组富



A: Flow cytometric analysis and quantification of cell cycle distribution in shControl and shLAMP2A SK-BR-3 cells; B: Flow cytometric analysis and quantification of cell cycle distribution in Control vector and pCMV6-LAMP2A SK-BR-3 cells; C: Western blot analysis and densitometric quantification of CDK4, CyclinD1, and CyclinE1 expression in shControl and shLAMP2A SK-BR-3 cells; D: Western blot analysis and densitometric quantification of CDK4, CyclinD1, and CyclinE1 protein expression in Control vector and pCMV6-LAMP2A SK-BR-3 cells. Cells in all groups were treated with 20 μmol/L carboplatin for 48 h

图5 卡铂对CMA活性不同的SK-BR-3细胞周期分布及细胞周期相关蛋白表达的影响 (n=3, $\bar{x}\pm s$)

Figure 5 Effects of carboplatin on cell cycle distribution and cell cycle-related protein expression in SK-BR-3 cells with different levels of CMA activity (n=3, $\bar{x}\pm s$)

集结果为进一步检测细胞周期分布及G1/S期相关蛋白提供了方向。但富集分析反映的是基因集合层面的相关性,尚不能单独证明CMA直接调控具体周期分子。

3.3 增强CMA可能通过维持细胞周期相关蛋白表达缓解G0/G1期阻滞

CDK4/CyclinD1复合物可促进RB蛋白磷酸化和E2F释放,CyclinE1与CDK2进一步推动DNA复制起始,是细胞完成G1/S期转换的重要分子基础[15-16]。在

HER2阳性乳腺癌中,CDK4/6相关通路还与肿瘤细胞增殖维持及治疗耐受密切相关[17-18]。本研究结果显示,卡铂处理后,敲低LAMP2A加重G0/G1期阻滞并降低CDK4、CyclinD1和CyclinE1表达;过表达LAMP2A减轻G0/G1期阻滞,并上调上述蛋白表达。细胞周期分布与周期蛋白表达的变化方向相互印证,支持增强CMA可能通过维持细胞周期轴活性,减轻卡铂诱导的G0/G1期阻滞并促进细胞进入S期。CMA既可能通过选择性降解细胞周期抑制因子直接影响周期推进,也可能通过缓解蛋白毒性、氧化应激和代谢压力间接维持周期蛋白表达[11, 28-29]。

3.4 不足与展望

本研究仍存在一定局限性。目前仅以HER2阳性乳腺癌SK-BR-3细胞为主要研究对象,尚需在其他HER2阳性乳腺癌细胞系、卡铂耐药细胞模型及体内模型中验证结论的普适性。另外,本研究主要从表达水平和细胞周期分布角度提示CMA与G1/S期转换相关,尚未直接证明CDK4、CyclinD1或CyclinE1为CMA的直接底物或关键下游节点,后续可结合CMA通量检测、蛋白质稳定性实验及功能救援实验进一步明确机制。

综上,LAMP2A介导的CMA活性增强可减轻卡铂对SK-BR-3细胞的增殖抑制,并与G0/G1期阻滞缓解、CDK4、CyclinD1和CyclinE1表达维持及G1/S期转换相关。上述结果提示,CMA-细胞周期轴可能参与HER2阳性乳腺癌细胞卡铂耐药,并为探索CMA靶向增敏策略提供实验依据。

利益冲突 本文所有作者声明不存在利益冲突

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