



CAMK2A通过激活ERK信号通路调控口腔鳞癌细胞 紫杉醇耐药性*

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【摘要】目的 研究钙离子信号通路调控口腔鳞癌(oral squamous cell carcinoma, OSCC)细胞紫杉醇耐药生存的分子机制。**方法** 通过紫杉醇低剂量持续诱导且剂量递增法诱导构建口腔鳞癌紫杉醇耐药细胞模型;使用Cell Counting Kit-8(CCK-8)和成像流式评估耐药模型的稳定性;通过RNA-seq测序分析差异基因和功能富集;使用实时荧光定量PCR(quantitative real-time PCR, qPCR)分析CAMK2a基因差异表达;通过蛋白质免疫印迹法(Western blot)检测蛋白质表达水平;用KN93及CAMK2a siRNA抑制CAMK2a表达。**结果** 成功构建紫杉醇耐药口腔鳞癌细胞模型;测序的GO富集结果显示耐药细胞在钙离子信号通路具有差异,其中CAMK2家族多种基因表达增加,CAMK2a分子表达增加12%($P < 0.05$);通过使用CAMK2a抑制剂KN93和siRNA基因敲低技术抑制CAMK2a后,耐药细胞增殖能力降低35%($P < 0.0001$),同时p-Erk1/2和下游分子表达降低。**结论** CAMK2a通过激活MAPK-Erk1/2通路,调控耐药细胞的耐药性和增殖能力,是临床耐药患者治疗的潜在新靶点。

【关键词】 口腔鳞癌 钙离子信号通路 紫杉醇耐药 CAMK2a 靶点

CAMK2a Regulates Paclitaxel Resistance in Oral Squamous Cell Carcinoma Cells by Activating the ERK Signaling Pathway

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[Abstract] Objective To investigate the molecular mechanisms by which calcium signaling pathways regulate the paclitaxel resistance and the survival of oral squamous cell carcinoma (OSCC) cells. **Methods** A paclitaxel-resistant OSCC cell model was established through continuous low-dose paclitaxel induction with dose escalation. The stability of the paclitaxel-resistant model was assessed using the Cell Counting Kit-8 (CCK-8) and imaging flow cytometry. RNA sequencing (RNA-seq) was performed to analyze differentially expressed genes and functional enrichment. Quantitative real-time PCR (qPCR) was conducted to analyze the differential expression of the CAMK2a gene. Protein expression levels were assessed by Western blot (WB). CAMK2a expression was inhibited using KN93 and CAMK2a small interfering RNA (siRNA). **Results** A paclitaxel-resistant OSCC cell model was successfully established. Gene Ontology (GO) enrichment analysis of the sequencing data revealed differences in the calcium signaling pathways in resistant cells. The expression of multiple genes of the CAMK2 family was upregulated, and CAMK2a expression increased by 12% ($P < 0.05$). After inhibiting CAMK2a by treatment with KN93, an inhibitor, and CAMK2a siRNA-mediated gene knockdown, the proliferative capacity of paclitaxel-resistant cells decreased by 35% ($P < 0.0001$). Furthermore, the expression levels of phosphorylated Erk1/2 (p-Erk1/2) and its downstream signaling molecules also decreased following CAMK2a inhibition. **Conclusion** CAMK2a regulates drug resistance and the proliferative capacity of paclitaxel-resistant cells by activating the MAPK-Erk1/2 pathway and represents a potential new therapeutic target for clinical treatment of drug-resistant patients.

[Key words] Oral squamous cell carcinoma Calcium signaling pathway Paclitaxel resistance
CAMK2a Target

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口腔鳞状细胞癌(oral squamous cell carcinoma, OSCC)是头颈部常见的恶性肿瘤之一,起源于口腔黏膜上皮。2022年全球新发病例约389 485例^[1-2]。目前治疗手段多样,但手术联合化疗仍是主要方案。然而,随着化疗疗程延长,多数患者逐渐产生耐药,导致预后不良。因此,深入探讨OSCC耐药机制,对于应对临床耐药现象、改善患者预后具有重要意义。

紫杉醇(paclitaxel, PTX)是一种经典的微管稳定剂类抗肿瘤药物,最初从太平洋红豆杉树皮中提取。作为一线化疗药物,紫杉醇通过多种机制诱导肿瘤细胞死亡,在头颈部鳞状细胞癌中,若患者无法耐受顺铂,常将紫杉醇作为替代治疗药物^[3-4]。在复发或转移性患者中,紫杉醇单药治疗的缓解率约为40%^[5]。然而,目前关于紫杉醇在OSCC中耐药机制的研究较少^[6],因此探索OSCC紫杉醇耐药的分子机制,对于改善顺铂不耐受患者的治疗结局具有重要临床意义。

化疗耐药的形成是多因素共同作用的结果,其中药物外排增强与肿瘤缺氧反应是两个较为经典的耐药机制。ABC转运蛋白家族是引起药物外排增强的重要分子^[7]。在肿瘤缺氧环境中,低氧诱导因子-1 α (hypoxia inducible factor-1 α , HIF-1 α)作为核心转录因子,可上调ABC转运蛋白家族的表达,从而参与耐药的形成^[8]。此外,已有研究表明钙离子信号变化与HIF-1 α 之间存在调控关系^[9-10]。以上研究提示,钙信号通路可能通过调控HIF-1 α /ABC转运蛋白轴,参与肿瘤细胞药物外排及耐药过程。

钙离子信号通路是一类通过钙离子作为第二信使调控细胞增殖、凋亡等生物学过程的重要信号通路。钙调蛋白(calmodulin, CAM)是一种高度保守的钙离子感受蛋白,通过结合Ca²⁺发生构象改变,进而激活或调节多种下游靶蛋白。其中,钙调蛋白依赖性蛋白激酶 II (Ca²⁺/Calmodulin-dependent protein kinase II, CAMK2)是CAM信号通路的重要下游效应分子。当细胞内钙离子浓度升高时,CAM与Ca²⁺复合体,进一步与CAMK2结合并诱导其自磷酸化,使CAMK2在钙信号消退后仍保持激活状态。研究表明,CAMK2突变不但与神经发育障碍相关,同时钙信号及CAMK2的异常也可能促进肿瘤的增殖、迁移和恶性进展^[11-13]。CAMK2的四个亚基(α 、 β 、 γ 、 δ)被认为可能与多种癌症的恶性表型和耐药性相关^[14],但其具体机制尚不明确。

基于上述研究背景,本研究构建了口腔鳞癌紫杉醇耐药细胞株,探讨CAMK2在耐药细胞中的作用机制,并评估通过调控CAMK2相关分子抑制肿瘤细胞增殖的策略,以期理解耐药机制和寻找治疗靶点提供依据。

1 材料与方法

1.1 材料

CAL27耐药细胞株由四川大学华西口腔医院构建;南美胎牛血清(FBS-PAN)购自成都宝生物科技有限公司;DMEM高糖培养基、Opti-MEM培养基购自美国Gibco公司;PBS缓冲液、CCK-8试剂盒及青霉素-链霉素双抗溶液(100 $\times$)购自兰杰柯科技有限公司;紫杉醇(HY-B0015)与KN93购自MedChemExpress公司;qPCR引物及siRNA由北京擎科生物科技股份有限公司合成,引物序列见表1;LipofectamineTM 2000转染试剂购自美国Invitrogen公司;Annexin V-EGFP/PI双染细胞凋亡检测试剂盒购自江苏凯基生物技术股份有限公司;抗体 β -actin(TA-09)及辣根过氧化物酶标记山羊抗兔IgG二抗购自北京中杉金

表 1 qPCR引物序列
Table 1 qPCR primer sequences

Gene	Primer sequences
BCRP	F (5'-3'): TGAGCCTACAACCTGGCTTAGA
	R (5'-3'): CCCTGCTTAGACATCCTTTTCAG
PKD2	F (5'-3'): AGTTCCTCATCAGCCAGATCC
	R (5'-3'): GATGCGAGCAAAGCCAAAGT
MRP1 (ABCC1)	F (5'-3'): CTCTATCTCTCCCCACATGACC
	R (5'-3'): AGCAGACGATCCACAGCAAAA
MRP2 (ABCC2)	F (5'-3'): CCATAGCTTCATTCCTGAGTAGC
	R (5'-3'): TCAGAGGACGCTTGAGCCTT
MRP3 (ABCC3)	F (5'-3'): CACCAACTCAGTCAAACGTGC
	R (5'-3'): GCAAGACCATGAAAGCGACTC
MRP4 (ABCC4)	F (5'-3'): GTGCTGGGATGAGGTTACGAG
	R (5'-3'): AAGGCACGATATTCCTATCTCCA
MRP5 (ABCC5)	F (5'-3'): ATCATGGCTTGAGTGCTCTGA
	R (5'-3'): AGACCACACGTCTTCCATTGA
MRP6 (ABCC6)	F (5'-3'): AGATGGTGCTTGATTGCGCC
	R (5'-3'): GCCACACAGTAGGATGAATGAG
MRP7 (ABCC10)	F (5'-3'): GTCCAGATTACATCCTACCCTGC
	R (5'-3'): GCCAACACCTCTAGCCCTATG
MRP8 (ABCC8)	F (5'-3'): GGTGACCGAATCCCACCATC
	R (5'-3'): CAGGGCAATTAGCAGCTTGG
MRP9 (ABCC12)	F (5'-3'): ATGATGCCGGGCTACTCTC
	R (5'-3'): CAGGGTGTCTACGGTCAGC
CAMK2A	F (5'-3'): GCTCTTCGAGGAATTGGGCAA
	R (5'-3'): CCTCTGAGATGCTGTCATGTAGT
CAMK2B	F (5'-3'): CTCTACGAGGATATTGGCAAGGG
	R (5'-3'): GCTTCTGGTGATCTCTGGCTG
CAMK2D	F (5'-3'): AGGGCTTTCACACTTGGTGT
	R (5'-3'): AGCCAAAGTCTGCCAATTTC
CAMK2G	F (5'-3'): ACCCGTTTCACCGACGACTA
	R (5'-3'): CTCCTGCGTGGAGGTTTTCTT

桥生物技术有限公司; 抗体GAPDH购自武汉三鹰生物技术有限公司; 抗体Erk1/2(ET1601)、p-Erk1/2(ET1610)、P-gp(HA500502)与Phospho-mTOR(HA600094)购自华安生物有限公司; 抗体MRP3(ab3375)与HIF-1 α (ab51608)购自Abcam公司; 抗体MRP1(72202s)购自美国Cell Signaling Technology公司; AceQ qPCR SYBR Green Master Mix购自南京诺唯赞生物科技股份有限公司; 细胞总RNA提取试剂盒购自成都福际生物技术有限公司; PrimeScriptTM FAST RT reagent Kit with gDNA Eraser试剂盒购自宝日医生物技术(北京)有限公司。

1.2 方法

1.2.1 细胞培养

CAL27细胞采用含10%胎牛血清及1%青霉素-链霉素双抗溶液的DMEM高糖培养基进行培养。

1.2.2 耐药细胞株构建

CAL27细胞培养至贴壁70%~80%时, 以40 nmol/L紫杉醇为初始浓度, 持续诱导至细胞正常生长, 更换完全培养基, 增加到45 nmol/L紫杉醇浓度再次诱导, 培养至细胞正常生长后再次增加紫杉醇浓度至50 nmol/L, 直至细胞可以在含50 nmol/L紫杉醇的培养基中稳定生长, 将此细胞命名为CAL27-PTX。

1.2.3 CCK-8检测细胞增殖

消化并收集对数生长期细胞, 以每孔 3×10^3 的细胞密度均匀接种于96孔板中。待细胞完全贴壁后, 将培养基更换为含不同药物浓度的新鲜培养基继续培养48 h; 去除培养基, 每孔加入CCK-8试剂, 37 °C孵育2 h, 酶标仪450 nm波长处测定吸光度(OD值)。细胞存活率采用(实验组OD值-空白组OD值)/(对照组OD值-空白组OD值) $\times 100\%$ 计算。

1.2.4 流式细胞术

消化收集经药物处理后的细胞, 使用预冷PBS(含1%胎牛血清)离心洗涤2次后, 按照细胞凋亡检测试剂盒使用说明进行细胞染色。染色完成后, 使用Amnis FlowSight成像流式细胞仪进行检测, 利用相应软件分析细胞凋亡率。

1.2.5 RNA提取、逆转录及qPCR

弃去细胞培养基, 使用预冷的PBS轻柔洗涤细胞一次后, 按照总RNA提取试剂盒说明书提取细胞总RNA。使用NanoDrop微量分光光度计检测RNA的纯度与浓度。取符合要求的RNA样品, 按照PrimeScriptTM FAST RT试剂盒说明书进行基因组DNA去除并逆转录合成cDNA。以cDNA为模板, 参照AceQ qPCR SYBR Green Master Mix试剂盒说明配制反应体系, 于实时荧光定量PCR仪上进行

扩增反应。所有基因的表达水平均采用 $2^{-\Delta\Delta C_t}$ 法进行计算分析。

1.2.6 RNA-seq测序

细胞总RNA的提取、文库构建及转录组测序由上海欧易生物医学科技有限公司完成, 并对原始测序数据进行基础生物信息学分析。

1.2.7 蛋白质免疫印迹(Western blot)

细胞经6孔板培养并处理后, 弃培养基, PBS洗涤。加入RIPA裂解液裂解细胞, 超声破碎后, 于4 °C、12 000 $\times g$ 离心10 min, 收集上清。BCA法测定蛋白浓度。蛋白样品与上样缓冲液混合, 100 °C煮沸10 min进行变性。随后进行SDS-PAGE电泳, 并转膜至PVDF膜。使用5%脱脂牛奶室温封闭1 h, 随后与一抗于4 °C孵育过夜。TBST洗膜后, 与相应二抗室温孵育1 h。最后使用超高敏ECL化学发光液显影, Image Lab软件采集图像并分析。

1.2.8 细胞转染

将细胞以约50%密度接种于6孔板中, 贴壁后更换为无血清DMEM培养基。按说明书, 分别用Opti-MEM稀释4 μ L的LipofectamineTM2000转染试剂及2 μ L的siRNA, 静置5 min后混合, 室温静置15 min以形成转染复合物。将复合物加入细胞培养液中, siRNA终浓度为40 nmol/L, 继续培养48 h后收集样本, 用于后续检测。

1.2.9 统计学方法

实验数据采用GraphPad Prism 9、Image Lab及ImageJ进行分析与图像处理。两组间单一数据比较使用*t*检验, $P < 0.05$ 为差异有统计学意义; CCK-8数据使用双因素重复测量方差分析, 其中细胞类型作为组间因素, 药物浓度作为重复测量因素, 检验水准控制采用Bonferroni法进行校正, $P < 0.05$ 为差异有统计学意义。测序数据富集结果的统计学意义根据分析软件输出的*P*值进行评估, 并采用FDR/*q*值进行多重检验校正。

2 结果

2.1 OSCC紫杉醇耐药细胞株CAL27-PTX的构建

实验结果显示, CAL27细胞经过长期、浓度梯度递增的紫杉醇诱导(图1A), 构建的口腔鳞癌紫杉醇耐药细胞株CAL27-PTX细胞可以在含50 nmol/L紫杉醇的培养液中稳定增殖。CCK-8结果(图1B)显示, CAL27-PTX细胞在紫杉醇处理后始终保持高于CAL27的细胞活性。双因素重复测量方差分析提示两种细胞的剂量反应模式差异有统计学意义[$F(5, 20) = 74.16, P < 0.0001$]。Bonferroni事后多重比较进一步显示, 除对照组(0 nmol/L PTX)外, 各浓度药物处理后的CAL27-PTX细胞活性均高于CAL27细

胞($P < 0.0001$)。流式细胞术结果(图1C)显示,与CAL27细胞相比,CAL27-PTX细胞对紫杉醇的耐受能力增强,高浓度(100 nmol/L)紫杉醇处理后,CAL27-PTX细胞仍维持较高的细胞活性,凋亡细胞比例降低。成像流

式实验(图1D)显示,CAL27-PTX细胞在紫杉醇刺激下呈现较弱的凋亡信号,进一步证明其具有抵抗紫杉醇诱导凋亡的能力。以上结果表明,OSCC紫杉醇耐药细胞株CAL27-PTX已成功构建。

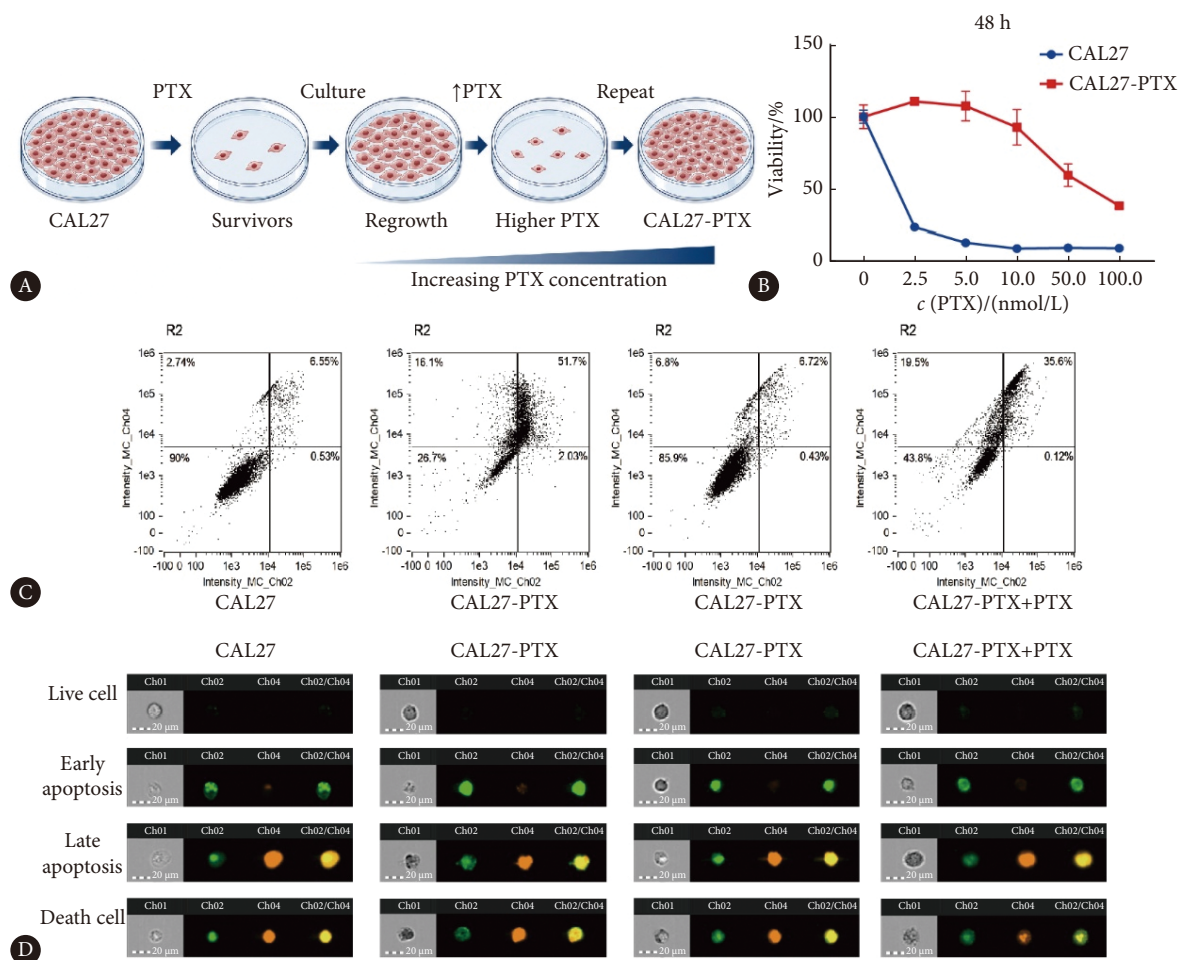


图1 紫杉醇耐药CAL27的构建和验证

Fig 1 Construction and validation of paclitaxel-resistant CAL27 cell lines

A, Schematic diagram of CAL27-PTX construction. B, Dose-response curves of CAL27 and CAL27-PTX cells. CAL27-PTX cells show higher viability than CAL27 cells at all PTX-treated concentrations. Two-way repeated measures ANOVA shows a significant interaction between drug concentration and cell type ($F[5, 20] = 74.16, P < 0.0001$). C, Flow cytometry analysis of apoptosis shows cell apoptosis and death in CAL27 and CAL27-PTX after treatment with 100 nmol/L paclitaxel for 48 h. D, Imaging flow cytometry shows weaker apoptotic signals in CAL27-PTX cells.

2.2 CAL27-PTX耐药基因表达升高

对CAL27细胞及紫杉醇耐药CAL27-PTX进行RNA-seq转录组测序分析结果显示,CAL27-PTX细胞中ABCC家族(主要编码多药耐药相关蛋白MRPs)的多个基因表达上升(图2A)。为验证此结果,我们通过qPCR实验检测了耐药相关基因的mRNA表达水平。结果显示,CAL27-PTX细胞中,耐药相关基因MRP1、MRP2、MRP3、MRP4、MRP5、MRP7、MRP8的表达水平高于CAL27细胞(图2B)。此外,与MRPs同属ABC转运蛋白超家族的BCRP(由ABCG2基因编码)以及和肿瘤恶性进展相关的

PKD2基因,在CAL27-PTX细胞中的表达均升高。

Western blot分析结果(图3)显示,在CAL27-PTX细胞中,缺氧诱导因子HIF-1 α 以及受到HIF-1 α 调控的ABC转运蛋白家族成员MRP1、MRP3和P-gp的表达水平均高于CAL27细胞。上述结果提示,紫杉醇耐药的OSCC细胞可能通过上调HIF-1 α 及多个药物外排泵关键蛋白,从而增强其对化疗药物紫杉醇的耐受能力。

2.3 CAL27-PTX细胞钙离子信号通路上调

为了探究耐药发生更上游的调控因素,我们对RNA-seq转录组测序结果进行GO与KEGG功能富集分析。其

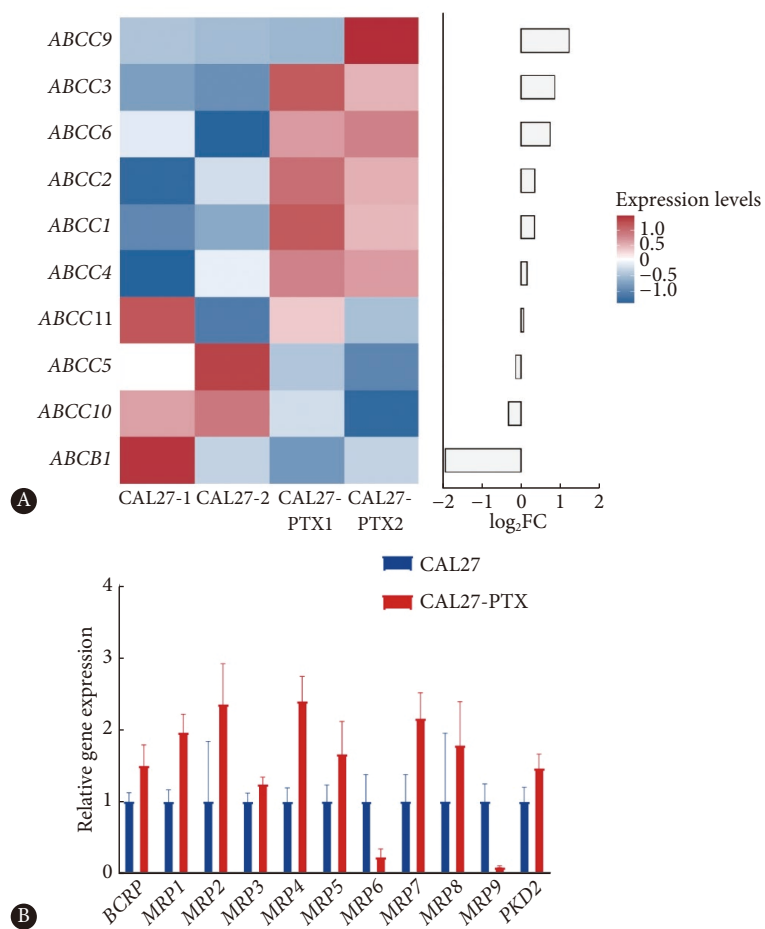


图 2 CAL27-PTX耐药基因高表达

Fig 2 High expression of drug resistance genes in CAL27-PTX

A, RNA sequencing shows elevated expression of drug resistance genes in CAL27-PTX; B, qPCR validation of drug resistance gene expression in CAL27-PTX.

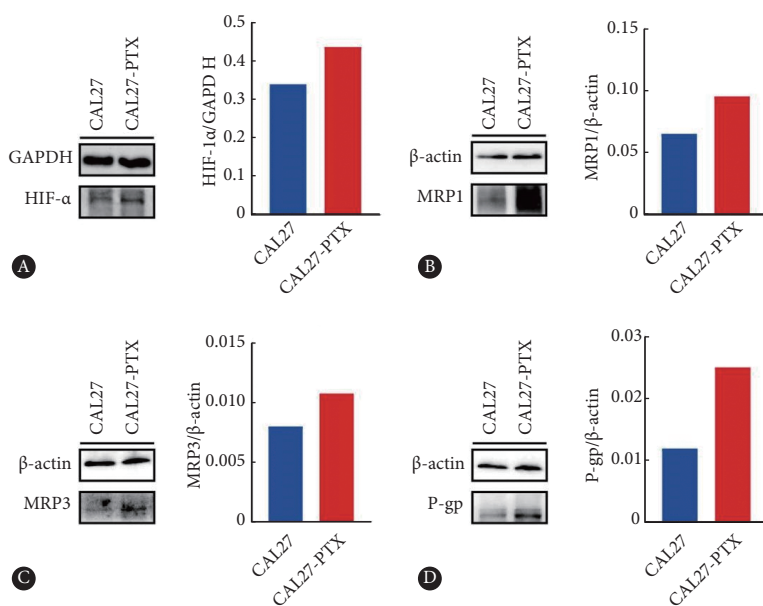


图 3 CAL27-PTX耐药蛋白高表达

Fig 3 High expression of drug resistance protein in CAL27-PTX

A-D, Representative Western blot bands and relative quantification of HIF-1α, MRP1, MRP3, and P-gp protein expression, respectively.

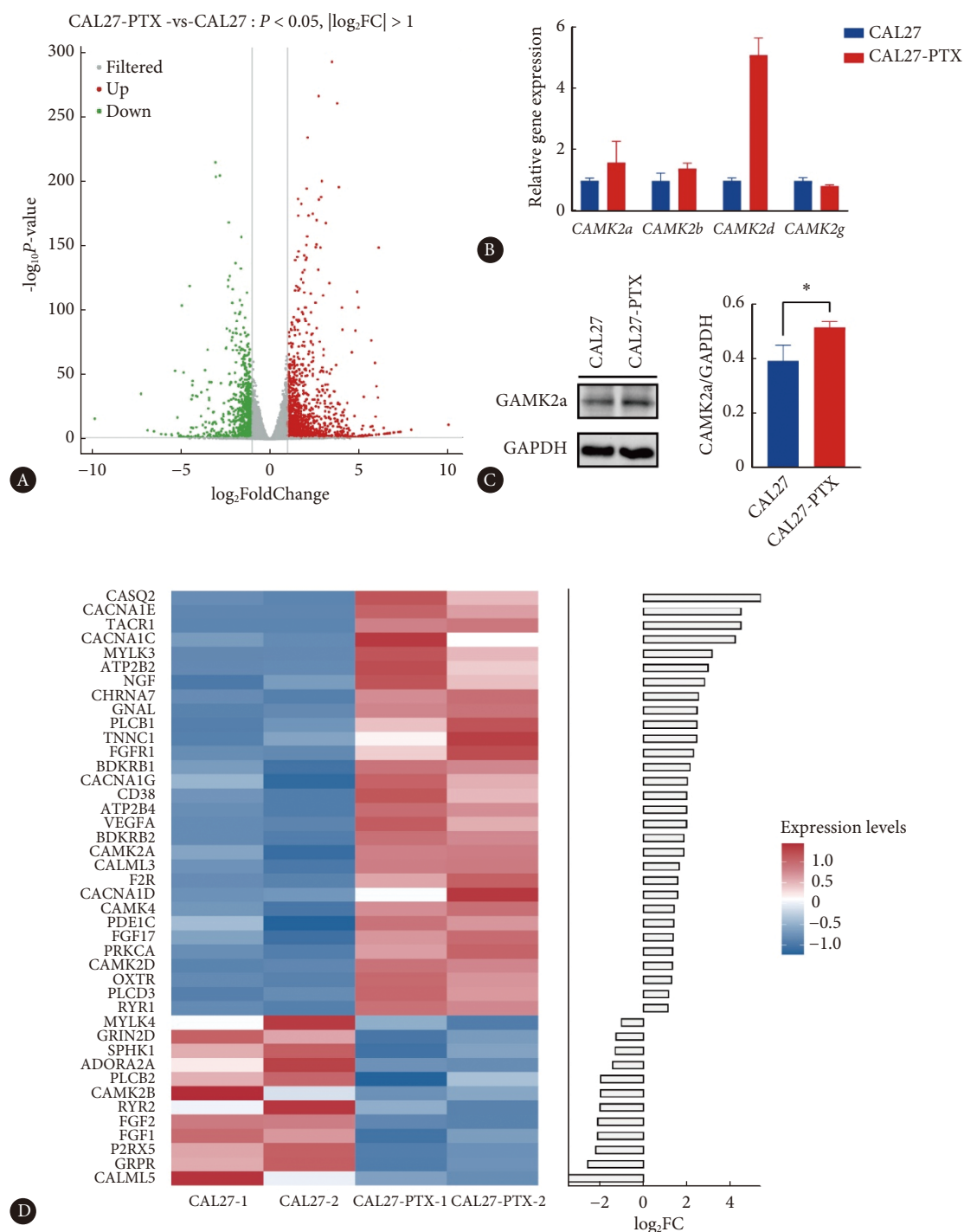


图 5 CAL27-PTX的CAMK家族蛋白具有明显差异

Fig 5 CAMK family proteins show significant differences in CAL27-PTX

A, Volcano plot of differentially expressed genes. Genes with $|\log_2FC| > 1$ and $FDR\text{-}adjq < 0.05$ were considered differentially expressed. B, qPCR validation of CAMK2 family gene expression differences. C, Western blot verification of CAMK2a upregulation in CAL27-PTX ($n = 3$, $P = 0.0254$). D, Heatmap of calcium signaling pathway-related differentially expressed proteins. The displayed proteins were selected based on calcium signaling pathway annotation and $FDR\text{-}adjq < 0.05$.

结果显示, CAMK2a在CAL27-PTX细胞中的表达亦高于CAL27细胞, 其相对表达量上调约12%, 差异具有统计学意义(图5D)。以上结果表明, CAMK2a表达上调可能与OSCC对紫杉醇的耐药性之间存在重要关联。

2.5 CAMK2a通过调控Erk1/2的磷酸化维持耐药细胞生长与增殖

为探究CAMK2功能, 我们采用其特异性抑制剂KN93处理CAL27-PTX细胞, KN93浓度根据MCE的

KN93体外研究示例进行设计,使用0~20 $\mu\text{mol/L}$ 的浓度处理CAL27-PTX细胞。如图6A所示,经梯度浓度KN93处理48 h后,CAMK2a的表达降低,其下游耐药相关信号通路的关键分子p-Erk1/2、p-mTOR与HIF-1 α 均受到抑制,而总Erk1/2水平未见明显变化。上述结果提示,CAMK2可能通过调控Erk1/2的磷酸化,进而影响其下游信号。CCK-8实验结果显示,KN93处理降低了CAL27-PTX的细胞活性,说明CAMK2可能参与调控耐药细胞Erk1/2的磷酸化维持耐药细胞的生长和增殖(图6B)。

为明确CAMK2a在调控Erk1/2磷酸化中的关键作用,我们利用siRNA在CAL27-PTX细胞中敲低CAMK2a的表达,使用siRNA进行转染敲低。随后采用Western blot验证敲低效率,结果显示si-CAMK2a具有较为明显的敲除效果(图6C),后续使用si-CAMK2a进行实验。Western blot结果显示,siRNA敲低使CAMK2a下降约20%后,p-Erk1/2水平亦随之降低,而总Erk1/2蛋白表达量未见明显变化,该结果与KN93抑制实验所获趋势一致(图6D,

6E)。进一步分析表明,下游p-mTOR与HIF-1 α 的表达水平亦相应下调。以上结果表明,CAMK2a可能是调控Erk1/2磷酸化并影响mTOR-HIF-1 α 信号轴、进而维持耐药细胞生长与增殖的关键亚基(图7)。

3 讨论

化疗耐药是导致肿瘤治疗失败的主要原因之一。多数肿瘤患者在化疗初期可获得一定程度的缓解,但随后的复发提示肿瘤已产生耐药性^[17]。对于OSCC患者,早期可通过手术单独治疗,但由于多数患者确诊时已处于晚期,需辅以放疗或全身治疗。目前可用于OSCC的化疗药物有限,因此克服耐药成为改善疗效的关键^[18]。深入研究OSCC耐药机制,对于制定更有效的治疗策略、改善患者预后具有重要意义。

药物外排能力增强是肿瘤耐药的重要特征之一。其中MRP1、MRP3、P-gp作为ABC转运蛋白家族成员,可通过促进化疗药物外排参与肿瘤耐药形成,这些转运蛋白

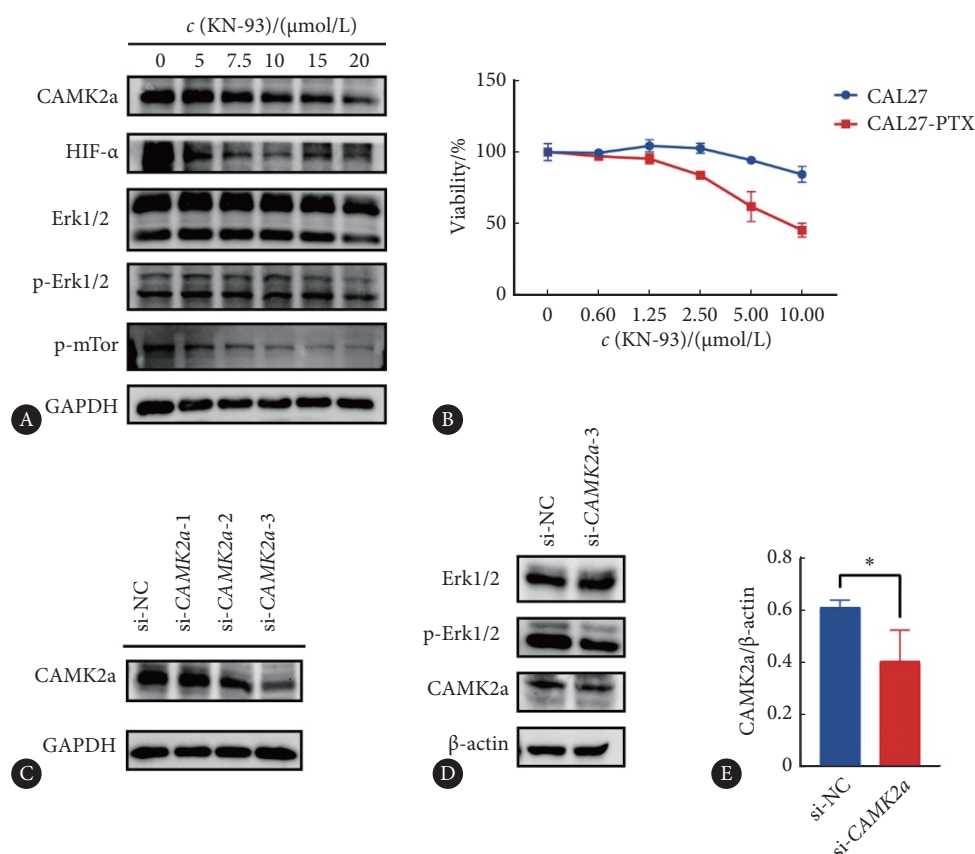


图6 CAMK2a调控Erk1/2磷酸化

Fig 6 CAMK2a regulates Erk1/2 phosphorylation

A, Western blot analysis shows changes in ERK-related proteins after CAMK2 inhibition by KN93. B, CCK-8 assay demonstrates that KN93 suppresses the viability of CAL27-PTX cells. Two-way repeated measures ANOVA shows a significant interaction between KN93 concentration and cell type ($F[5, 20] = 17.31, P < 0.0001$). Bonferroni-adjusted comparisons show statistically significant differences at the fourth to sixth concentrations. C, Validation of the effect of CAMK2a siRNA. D, Expression of related proteins after siRNA-mediated knockdown of CAMK2a. E, Changes in CAMK2a expression following siRNA inhibition ($n = 3, P = 0.043$).

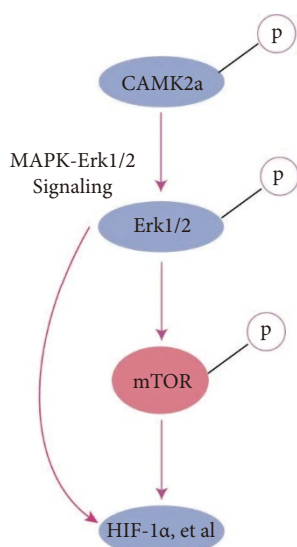


图 7 CAMK2a的下游调控机制

Fig 7 Downstream regulatory mechanism of CAMK2a

的功能可受缺氧诱导因子HIF-1 α 的调节,而HIF-1 α 表达又与细胞内钙离子水平的变化相关^[9-10]。因此,我们推测钙离子信号通路可能在耐药调控中发挥关键作用。

钙调蛋白依赖性蛋白激酶CAMK2是钙离子信号通路中钙调蛋白的下游靶点。在接收到钙信号后,CAMK2被激活,发生磷酸化,参与调控神经突触可塑性、学习记忆以及心肌收缩等生理过程^[19]。研究表明,CAMK2蛋白家族成员在多种肿瘤中存在过表达或异常激活^[20]。CAMK2a作为CAMK2分子的亚基,除参与调节多种神经元功能外^[21-22],近年研究发现其与肿瘤耐药存在一定关联^[16]。Erk1/2通路是丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)级联反应的重要分支,Erk1/2分子在被双磷酸化后被激活后转入细胞核,磷酸化多种转录因子,从而调控细胞增殖、分化、存活与迁移等。在肿瘤中,该通路常处于异常激活状态,促进细胞无限增殖,抑制凋亡,并可上调HIF-1 α 表达,增强肿瘤耐药性^[23-24]。研究表明,使用CAMK2抑制剂KN-93治疗后,皮质神经元中Erk磷酸化被阻断^[25-26]。基于上述证据,我们推测CAMK2a可能通过调控ERK磷酸化,影响下游基因表达和细胞存活,从而参与OSCC的耐药过程。

本课题组前期已建立OSCC紫杉醇耐药细胞系,并验证了耐药相关基因的表达变化。通过RNA-seq测序及差异表达分析,筛选出紫杉醇耐药细胞中显著差异表达的基因。在已有研究提示钙离子信号通路可能参与肿瘤耐药调控的基础上^[27-29],本研究聚焦CAMK家族,并最终选定CAMK2a作为研究对象。结果发现,CAMK2a在耐药细胞中通过维持Erk1/2的磷酸化激活,促进下游信号通路的传导,从而支持细胞生存与增殖。抑制CAMK2a可

有效抑制耐药细胞的增殖,提示CAMK2a可能作为OSCC紫杉醇耐药的潜在治疗靶点。

* * *

作者贡献声明 王俊峰负责论文构思、数据审编、正式分析、调查研究、研究方法、研究项目管理、可视化和初稿写作,陈红利负责论文构思、数据审编、正式分析、调查研究、提供资源、监督指导和审读与编辑写作,陈娇负责论文构思、经费获取、提供资源和监督指导,冯云负责研究项目管理和监督指导,袁尧负责提供资源和审读与编辑写作,张平负责经费获取、研究项目管理、提供资源、监督指导和审读与编辑写作。所有作者已同意将文章提交至本刊,并对将要发表版本进行最终定稿,且同意对工作的所有方面负责。

Author Contribution Wang Junfeng is responsible for conceptualization, data curation, formal analysis, investigation, methodology, project administration, visualization, and writing--original draft. Chen Hongli is responsible for conceptualization, data curation, formal analysis, investigation, resources, supervision, and writing--review and editing. Chen Jiao is responsible for conceptualization, funding acquisition, resources, and supervision. Feng Yun is responsible for project administration and supervision. Yuan Yao is responsible for resources and writing--review and editing. Zhang Ping is responsible for funding acquisition, project administration, resources, supervision, and writing--review and editing. All authors consented to the submission of the article to the Journal. All authors approved the final version to be published and agreed to be accountable for all aspects of the work.

利益冲突 所有作者均声明不存在利益冲突

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