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在线全文

胃癌中PCMT1表达的预后价值及其对纺锤体组装检查点的调控作用*

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【摘要】 目的 探讨蛋白质-L-异天冬氨酸(D-天冬氨酸)-甲基转移酶(PCMT1)在胃癌中的表达及对预后的影响，并分析其潜在的作用机制。**方法** UALCAN数据库在线分析PCMT1在胃癌组织的表达情况，通过DAVID数据库进行基因本体注释(GO)及京都基因和基因组百科全书(KEGG)信号通路富集分析其可能的功能与信号通路。纳入2014年1月–2017年12月于我院接受胃癌根治术的120例患者，免疫组织化学染色检测PCMT1、Ki67在胃癌组织中的表达；Cox回归、Kaplan-Meier曲线和受试者工作特征(ROC)曲线用于胃癌患者术后5年生存率的预后分析。采用慢病毒构建干扰或过表达PCMT1载体，转染人胃癌细胞系MGC-803与HGC-27细胞，设置干扰空载体(sh-NC)组与干扰PCMT1载体(sh-*PCMT*1)组，过表达空载体(LV-Vec)组与过表达PCMT1载体(LV-*PCMT*1)组；Western blot检测各组细胞中PCMT1、CyclinB1、CDC20蛋白表达，CCK-8法检测胃癌细胞增殖能力，流式细胞术检测细胞周期。注射4组MGC-803细胞构建裸鼠皮下移植瘤模型，每组3只，测量体质量，14 d后处死裸鼠，测量肿瘤体积，Western blot法检测肿瘤组织中CyclinB1与CDC20蛋白表达水平。**结果** UALCAN数据库分析示PCMT1在胃癌组织中高表达且在不同病理分期、分级与淋巴结转移的胃癌组织中均表达升高(P<0.05)。GO与KEGG富集示PCMT1主要参与有丝分裂、纺锤体组装检查点与细胞周期等信号。免疫组化结果显示PCMT1与Ki67在患者胃癌组织中呈高表达且二者呈正相关关系(P<0.05)。Cox多因素分析示PCMT1高表达〔风险比(HR)=2.921，95%置信区间(CI)：1.628~5.239〕是影响胃癌患者术后5年生存率的独立危险因素之一。Kaplan-Meier曲线分析示高表达PCMT1的患者术后5年生存率(16.7%，HR=4.651，95%CI=2.846~7.601)低于低表达PCMT1患者(70.0%，HR=0.215，95%CI=0.132~0.351)。ROC曲线表明，PCMT1预测患者术后5年生存率的曲线下面积为0.764(95%CI：0.674~0.854)。Western blot对PCMT1的检测结果表明慢病毒干扰或过表达PCMT1细胞系构建成功。CCK-8结果表明下调PCMT1表达MGC-803与HGC-27细胞增殖能力减弱，过表达PCMT1则促进细胞增殖(P<0.05)。干扰PCMT1后CDC20蛋白表达下调，CyclinB1蛋白表达上升，细胞周期阻滞于G_2/M期，而过表达则呈现相反趋势(P<0.05)。sh-*PCMT*1组裸鼠肿瘤的体积与质量减小，肿瘤组织CDC20蛋白表达降低，CyclinB1蛋白表达升高(P<0.05，与sh-NC组相比)，LV-*PCMT*1组则呈相反趋势(P<0.05，与LV-Vec组相比)。**结论** PCMT1在胃癌组织中呈高表达，与患者不良预后相关，可能通过调控细胞有丝分裂进程中纺锤体检查点影响肿瘤细胞恶性增殖。

【关键词】 胃癌 PCMT1 预后 纺锤体组装检查点

Prognostic Value of PCMT1 Expression in Gastric Cancer and Its Regulatory Effect on Spindle Assembly Checkpoints

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【Abstract】 Objective The study was conducted to investigate the expression of protein-L-isoaspartate (D-aspartate) O-methyltransferase (PCMT1) in gastric cancer and its effect on the prognosis, and to analyze its potential mechanism. **Methods** UALCAN, a cancer data analysis platform, was used to conduct online analysis of the expression

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of PCMT1 in gastric cancer tissues. Through the Database for Annotation, Visualization and Integrated Discovery (DAVID), Gene Ontology (GO) annotation and signaling pathway enrichment by Kyoto Encyclopedia of Genes and Genomes (KEGG) were performed to analyze the possible functions and signaling pathways. A total of 120 patients who underwent radical gastrectomy for gastric cancer between January 2014 and December 2017 in our hospital were enrolled for the study. Immunohistochemical staining was performed to determine the expression of PCMT1 and Ki67 in gastric cancer tissues. Cox regression, Kaplan-Meier curve, and receiver operating characteristic (ROC) curves were used for prognostic analysis of 5-year survival in gastric cancer patients after surgery. Lentivirus was used to construct PCMT1-interfering or PCMT1-overexpressing vectors, which were then used to transfect human gastric cancer cell lines of MGC-803 and HGC-27 cells. The interfering empty vector (sh-NC) group, the interfering PCMT1 vector (sh-*PCMT1*) group, the overexpressing empty vector (LV-Vec) group, and the overexpressing PCMT1 vector (LV-*PCMT1*) group were set up. Western blot was performed to determine the protein expression levels of PCMT1, CyclinB1, and CDC20. CCK-8 assay was performed to measure the proliferation of gastric cancer cells. Flow cytometry was performed to determine the cell cycle. MGC-803 cells were injected in four groups of nude mice to construct a subcutaneous xenograft tumor model, with three nude mice in each group. The body mass of the nude mice was measured. The nude mice were sacrificed after 14 days and the tumor volume was monitored. The expression levels of CyclinB1 and CDC20 proteins in the tumor tissues were determined by Western blot assay. **Results** Analysis with UALCAN showed that PCMT1 was highly expressed in gastric cancer tissues. Moreover, elevated expression was found in gastric tumor tissues of different pathological stages and grades and those with lymph node metastasis (P<0.05). GO and KEGG enrichment analyses showed that PCMT1 was mainly involved in the signal regulation of mitosis, spindle assembly checkpoints, and cell cycle. The immunohistochemical results showed that PCMT1 and Ki67 were highly expressed in gastric cancer tissues and that they were positively correlated with each other (P<0.05). Cox multivariate analysis showed that high PCMT1 expression (hazard ratio [*HR*]=2.921, 95% confidence interval [*CI*]:1.628-5.239) was one of the independent risk factors affecting the 5-year survival rate of gastric cancer patients after surgery. Kaplan-Meier curve showed that patients with high PCMT1 expression had a lower 5-year survival after surgery (16.7%, *HR*=4.651, 95% *CI*: 2.846-7.601) than patients with low PCMT1 expression (70.0%, *HR*=0.215, 95% *CI*: 0.132-0.351) did. The ROC curve showed that PCMT1 had an area under the curve (*AUC*) of 0.764 (95% *CI*: 0.674-0.854) for predicting 5-year patient survival after surgery. Western blot results showed that lentiviral interference or overexpression of PCMT1 cell lines was successfully constructed. The results of CCK-8 showed that the proliferative ability of MGC-803 and HGC-27 cells was weakened with the downregulation of PCMT1, and the overexpression of PCMT1 promoted cell proliferation (P<0.05). With the interference of PCMT1, the expression of CDC20 protein was decreased, the expression of CyclinB1 protein was increased, and the cell cycle was arrested in the G_2/M phase. In contrast, the overexpression of PCMT1 led to the opposite trends (P<0.05). In the sh-*PCMT*1 group, the tumor volume and mass were decreased and the expression of CDC20 protein was decreased and the expression of CyclinB1 protein was increased in the tumor tissues of the nude mice (P<0.05, compared with those of the sh-NC group. In contrast, the LV-*PCMT*1 group showed the opposite trends (P<0.05, compared with those of the LV-Vec group). **Conclusion** The high expression of PCMT1 in gastric cancer tissues is associated with poor prognosis in patients and may affect tumor cell malignant proliferation via regulating spindle checkpoints in the process of mitosis.

【Key words】 Gastric cancer PCMT1 Clinical prognosis Spindle assembly checkpoint

胃癌作为常见消化道恶性肿瘤，居于全球癌症相关死亡原因的第三位[1]。近年来以手术为主的综合治疗虽取得显著成效，但患者术后5年生存率仍然处于较低水平[2]。尽管越来越多的分子与病理研究逐步揭示胃癌的发生过程，但其复杂的分子机制仍未完全阐明[3-4]。癌细胞在有丝分裂过程中具有染色体不稳定性特征，导致一个或多个细胞周期检查点发生异常，其中涉及纺锤体组装检查点(spindle assembly checkpoint, SAC)[5]。蛋白质-L-异天冬氨酸(D-天冬氨酸)-甲基转移酶(protein D-aspartate/L-isoaspartate methyltransferase, PCMT1)作为编码Ⅱ型蛋白质羧基甲基转移酶成员，负责异构化天冬氨酸残基转换为正常结构，在蛋白质修复中发挥重要作用[6-7]。近年来研究发现，PCMT1在肺癌、膀胱癌和乳腺癌中发挥癌基因的作用，其高表达提示预后不良[8-10]。然而，PCMT1在胃癌中的作用、预后价值及潜在的分子机制尚未见报道。本研究基于胃癌患者的临床病例资料及公共数据库，结合分子病理学、分子生物学与生物信息学技术，探讨胃癌中PCMT1的表达情况，分析其对胃癌患者远期预后的影响，进而探究其对胃癌细胞恶性增殖的影响及可能的作用机制，旨在为胃癌的治疗和预后提供潜

在的分子靶点。

1　资料与方法

1.1　一般资料

回顾性分析2014年1月–2017年12月于蚌埠医学院第一附属医院接受胃癌根治术的患者。纳入标准: 经临床与病理学诊断为原发性胃癌; 成功施行胃癌根治术并达到R0切除。排除标准: 合并其他组织起源的恶性肿瘤; 临床资料缺失, 术后死于胃癌以外的其他因素。依据纳入及排除标准入选并采集患者的相关资料: 一般临床资料, 通过电子病历系统采集患者性别、年龄、肿瘤临床分期、手术病理诊断、肿瘤标志物等数据; 生存资料, 电话随访采集患者术后5年生存情况及死亡原因; 手术病理蜡块, 调取病理科胃癌和癌旁组织蜡块, 进行免疫组织化学染色。最终纳入120例。该研究通过蚌埠医学院第一附属医院伦理委员会批准, 批准号: 伦科批字〔2021〕第221号。

1.2　材料与试剂

人胃癌细胞系MGC-803购自中国科学院典型培养物保藏委员会细胞库, 人胃癌细胞系(未分化)HGC-27购自武汉普诺赛生命科技有限公司; 胎牛血清(FBS)购自美国Gibco公司; 双抗、DMEM高糖培养基购自北京索莱宝公司; RNA逆转录试剂盒及SYBR Green购自大连Takara公司; 蛋白质提取与定量试剂盒、ECL化学发光液、细胞周期检测试剂盒购自上海碧云天公司。兔抗人GAPDH、PCMT1、CyclinB1、CDC20、Ki67抗体及酶标二抗等购自武汉Proteintech或美国Abcam公司; 引物由南京金斯瑞公司合成。

1.3　方法

1.3.1　生物信息学分析

UALCAN数据库纳入449例样本分析PCMT1在胃癌中的表达情况, cBioPortal数据库获取胃癌与PCMT1的相关基因后, 通过DAVID数据库进行基因本体注释(GO)及京都基因和基因组百科全书(KEGG)信号通路富集分析其可能的功能与信号通路。

1.3.2　免疫组织化学染色检测PCMT1、Ki67在胃癌组织中的表达

将手术病理蜡块制成5 μm厚度切片, 依次进行烤片、脱蜡水化、抗原修复、阻断内源性过氧化物酶活性、封闭、PCMT1(1∶200, Proteintech)或Ki67(1∶400, Abcam)一抗孵育、二抗孵育、DAB显色、细胞核复染、脱水透明、封片及采集图片。评估方法: 由两位病理科医师对切片进行分析, 每张切片于高倍镜下(×200)选取5个不重复视野, 采用Image软件分析目标蛋白相对积分光密度值(integrated option density, IOD), 取平均值作为最终结果。

1.3.3　细胞培养、转染及分组

为探究PCMT1对肿瘤细胞的影响, 采用慢病毒过表达及干扰PCMT1载体转染MGC-803与HGC-27细胞株。PCMT1下调实验设置干扰空载体组(sh-NC)与干扰PCMT1载体组(sh-*PCMT*1), PCMT1过表达实验设置过表达空载体组(LV-Vec)与过表达PCMT1载体组(LV-*PCMT*1)。按实验分组转染不同的病毒原液。此处的病毒原液是加入了慢病毒表达载体和包装质粒, 经转染、包装等过程制备出滴度为1×10^8 TU/mL的病毒原液, 以进行后续细胞转染实验。具体为:

将MGC-803与HGC-27细胞置于含10%FBS的完全培养基与37 ℃、体积分数5%CO_2培养箱中进行持续培养, 并取对数生长期细胞进行后续实验。0.25%胰酶消化并制成细胞悬液, 接种于96孔板, 待细胞汇合度达70%时对细胞进行转染, 依据MOI值计算需要加入的病毒载量。每孔中加入2 μL的1×10^8 TU/mL病毒原液, 置于培养箱中继续培养12 ~ 16 h后, 更换为完全培养基, 并加入2 μg/mL嘌呤霉素筛选稳定转染的细胞株。转染5 d后收集细胞, Western blot法检测PCMT1蛋白表达情况。具体检测方法见1.3.4。

1.3.4　Western blot实验检测MGC-803与HGC-27细胞中PCMT1、CyclinB1、CDC20蛋白表达

取MGC-803与HGC-27细胞或裸鼠肿瘤组织, RIPA裂解细胞悬液制成总蛋白, BCA法测定总蛋白浓度, 配制10%分离胶与5%浓缩胶, 依次经电泳、转膜、封闭、一抗孵育4 ℃过夜、二抗孵育、ECL化学发光试剂盒显色, Bio-Rad仪器曝光条带, Image软件分析蛋白灰度值。一抗信息如下: PCMT1(1∶1 000, Proteintech)、CyclinB1(1∶1 000, Proteintech)、CDC20(1∶1 000, Proteintech)、内参蛋白GAPDH(1∶3000, Abcam)。以目的条带灰度值与内参蛋白条带灰度值的比值为目的条带的相对表达量, 再设对照组(sh-NC组或LV-Vec组)的目的蛋白表达量的均数为1, 计算其他组相对于对照组目的蛋白的表达。

1.3.5　CCK-8实验检测MGC-803与HGC-27细胞的增殖能力

依据实验分组, 将4组细胞消化制成悬液, 以1×10^3细胞密度接种于96孔板, 分别于24 h、48 h与72 h加入含10%CCK-8试剂的100 μL培养基, 使用酶标仪于450 nm处测定吸光度值。

1.3.6　流式细胞术检测MGC-803与HGC-27细胞的细胞周期

依据实验分组, 胰酶消化4组细胞并收集细胞悬液, 经PBS洗涤、离心、70%预冷乙醇4 ℃固定过夜、碘化丙

啶与RNaseA染色，37 ℃避光孵育30 min，流式细胞仪检测各个时期细胞比例。

1.3.7 裸鼠皮下成瘤实验

BALB/c-nude裸鼠购自江苏集萃药康生物科技股份有限公司，均为6周龄雄鼠，(20±2) g，每组3只，共12只，饲养于无特定病原体动物(SPF)级环境下。取对数生长期细胞(MGC-803)，0.25%胰酶消化并制成细胞悬液，将100 μL(1×10^8 mL^{-1})的细胞(sh-NC组、sh-*PCMT*1组、LV-Vec组与LV-*PCMT*1组)悬液接种于裸鼠背部皮下，于接种后于第4 天、第8天、第12天、第14天观察并记录肿瘤体积，肿瘤体积=(长径×短径^2)/2。肿瘤体积以 mm^{3}为单位。于接种后第14天处死裸鼠，取肿瘤并拍照称重。收集肿瘤组织并采用Western blot法检测组织中CyclinB1与CDC20蛋白表达水平，方法见1.3.4。本实验通过蚌埠医学院动物伦理委员会批准，批准号：伦动科批字[2022]第283号。

1.3.8 统计学方法

计量资料以$\bar{x}\pm s$表示，两独立样本之间比较(sh-NC组与sh-*PCMT*1比较，LV-Vec与LV-*PCMT*1比较)采用重复测量的双因素方差分析、*t*检验；ULCAN数据库分析中的原始数据以中位数、四分位间距表示的数据资料采用非参数检验。χ^2检验用于率的比较。Spearman检验用于相关性分析，Kaplan-Meier曲线分析术后5年生存率，Cox回归分析影响患者术后5年生存率的影响因素，受试者工作特征(ROC)曲线分析PCMT1对胃癌患者术后5年生存率的预判价值。

2 结果

2.1 PCMT1在胃癌中的表达特征及GO功能注释与KEGG通路富集

UALCAN数据库分析显示(表1)，相比于正常组织，PCMT1在胃癌组织中呈高表达(P<0.001)。不同肿瘤分级、病理分期及淋巴结转移的胃癌组织中PCMT1的表达均高于正常组织(P<0.05)。此外，GO分析显示，PCMT1可能参与的生物学过程涉及细胞分裂、DNA修复、姐妹染色单体分离等，细胞组分主要包括核质、着丝粒、线粒体等，分子功能主要富集在RNA、蛋白质、ATP结合等条目。KEGG富集分析显示，PCMT1主要参与CDC20介导的有丝分裂、纺锤体组装检查点、细胞周期等信号的调控。

2.2 PCMT1在胃癌组织中高表达

免疫组织化学结果显示，胃癌患者病理组织切片中，PCMT1主要表达于癌细胞胞质中，Ki67主要表达于癌细胞胞核；在胃癌组织中的PCMT1(相对IOD值：2.95±1.22 vs. 1.10±0.16)与Ki67(相对IOD值：3.92±1.05 vs. 1.06±0.18)相比癌旁组织呈高表达(P<0.05，图1)；相关性分析示，PCMT1与Ki67在胃癌组织中的表达量呈正相关(r=0.629，P<0.01)。

表 1 UALCAN数据库分析PCMT1在胃癌中的表达情况

Table 1 Analysis of PCMT1 expression in gastric cancer with UALCAN, a cancer data analysis platform

Characteristic	Case (n=449)	Transcript per million (median, P_{25}-P_{75})	P
Sample type			<0.001
Normal	34	46.740, 37.455-53.657	
Primary tumor	415	57.811, 48.148-70.690	
Tumor grade			
Normal	34	46.740, 37.455-53.657	
Grade 1	12	50.970, 46.457-65.687	0.021*
Grade 2	148	61.270, 48.378-72.860	<0.001*
Grade 3	246	56.565, 47.998-69.698	<0.001*
Individual cancer stage			
Normal	34	46.740, 37.455-53.657	
Stage 1	18	60.022, 48.005-70.115	0.016*
Stage 2	123	57.689, 48.248-71.373	<0.001*
Stage 3	169	56.683, 48.519-67.456	<0.001*
Stage 4	41	67.857, 47.830-89.244	<0.001*
Nodal metastasis status			
Normal	34	46.411, 38.023-53.469	
N0	123	58.425, 48.127-69.764	<0.001*
N1	112	59.160, 49.649-72.101	<0.001*
N2	79	56.139, 47.965-70.834	<0.001*
N3	82	56.284, 46.573-66.432	<0.001*

* In comparison with that of normal sample.

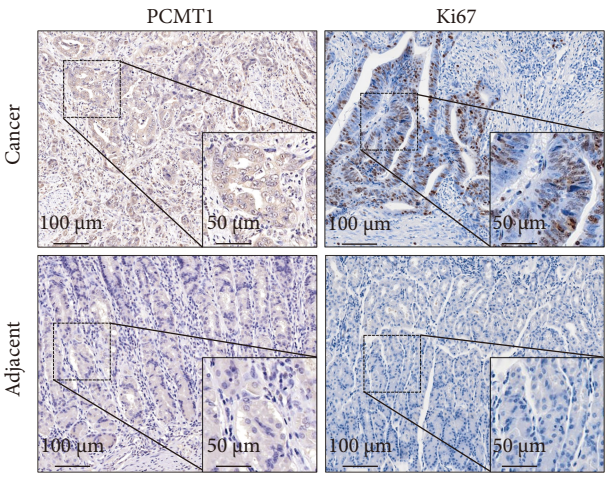

图 1 PCMT1在胃癌组织中的表达（免疫组化染色）

Fig 1 Expression of PCMT1 in gastric cancer tissues (immunohistochemistry staining)

2.3 PCMT1的表达与胃癌进展和临床病理分期相关

以PCMT1相对表达量的中位数(2.97)为界，将患者分为PCMT1高表达组(n=60)和PCMT1低表达组(n=60)。如表2，PCMT1高表达组患者G3-4分级、T3-4分

表 2　胃癌组织中PCMT1的表达与临床病理参数间的关系

Table 2　Association of PCMT1 expression level with the clinicopathological parameters in gastric cancer tissues

Factor	Total	PCMT1/case (%)		χ^2	*P*
		Low expression (*n*=60)	High expression (*n*=60)		
Sex				2.342	0.126
Male	93	43 (46.2)	50 (53.8)		
Female	27	17 (63.0)	10 (37.0)		
Age/yr.				0.037	0.847
<60	41	20 (48.8)	21 (51.2)		
⩾60	79	40 (50.6)	39 (49.4)		
Tumor size/cm				3.429	0.064
<5	50	30 (60.0)	20 (40.0)		
⩾5	70	30 (42.9)	40 (57.1)		
Cancer cell type				2.160	0.142
Adenocarcinoma	100	53 (53.0)	47 (47.0)		
Other*	20	7 (35.0)	13 (65.0)		
CEA/(μg/L)				4.821	0.028
<5	64	38 (59.4)	26 (40.6)		
⩾5	56	22 (39.3)	34 (60.7)		
CA19-9/(U/L)				4.062	0.044
<37 000	65	38 (58.5)	27 (41.5)		
⩾37 000	55	22 (40.0)	33 (60.0)		
Pathological grading				22.558	<0.001
G1-G2	62	44 (71.0)	18 (29.0)		
G3-G4	58	16 (27.6)	42 (72.4)		
T stage				4.800	0.028
1-2	60	36 (60.0)	24 (40.0)		
3-4	60	24 (40.0)	36 (60.0)		
N stage				6.541	0.011
0-1	62	38 (61.3)	24 (38.7)		
2-3	58	22 (37.9)	36 (62.1)		

* Other classifications in cancer cell types include signet ring cell carcinoma and mucinous adenocarcinoma.

期、N2-3分期、CEA⩾5 μg/L与CA19-9⩾37 000 U/L的比例高于低表达组(*P*<0.05)，而两组患者的性别、年龄、肿瘤大小与组织类型差异无统计学意义(*P*>0.05)。

2.4　PCMT1表达对胃癌患者根治术后5年生存率的影响

Kaplan-Meier生存分析显示，PCMT1高表达组的胃癌患者术后5年生存率〔16.7%，风险比(*HR*)=4.651，95%置信区间(*CI*)：2.846~7.601〕低于低表达组(70.0%，*HR*=0.215，95%*CI*：0.132~0.351，Log-rank χ^2=39.688，*P*<0.001，图2)。

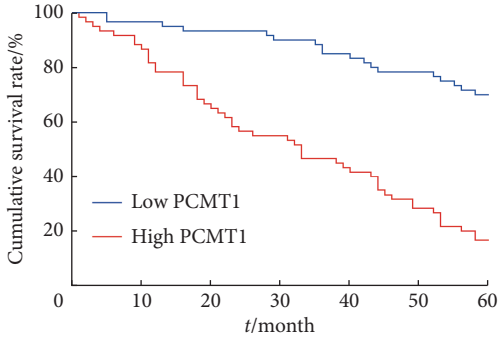

图 2 PCMT1的表达与胃癌患者根治术后5年生存率的关系

Fig 2　Relationship between PCMT1 expression and 5-year survival of the gastric cancer patients after radical gastrectomy

2.5　影响胃癌根治术后5年生存率的单因素与多因素分析

单因素分析显示，PCMT1高表达、CEA⩾5 μg/L、CA19-9⩾37 000 U/L、G3-4级、T3-4期、N2-3期可能是影响胃癌患者术后5年生存率的危险因素。Cox回归模型分析示，PCMT1高表达(*HR*=2.921，95%*CI*：1.628~5.239)、CEA⩾5 μg/L、G3-4级、T3-4期、N2-3期可能是影响胃癌患者术后5年生存率的危险因素(表3，*P*<0.05)。

2.6　PCMT1对胃癌患者术后5年生存率具有预判价值

以PCMT1预判胃癌患者根治术后5年生存率的曲线

表 3　影响胃癌根治术后5年生存率的单因素与多因素分析

Table 3　Univariate and multivariate analyses of 5-year survival of the gastric cancer patients after radical gastrectomy

Factor	Univariate analysis		Multivariate analysis		
	Log-rank χ^2	*P*	*HR*	95% *CI*	*P*
Sex (female vs. male)	1.183	0.277	–	–	–
Age (⩾60 yr. vs. <60 yr.)	1.880	0.170	–	–	–
Tumor size (⩾5 cm vs. <5 cm)	3.364	0.067	–	–	–
Cancer cell type (adenocarcinoma vs. other)	1.381	0.240	–	–	–
PCMT1 expression (high vs. low)	39.688	<0.001	2.921	1.628-5.239	<0.001
CEA (⩾5 μg/L vs. <5 μg/L)	25.977	<0.001	2.908	1.724-4.904	<0.001
CA19-9 (⩾37 000 U/L vs. <37 000 U/L)	21.811	<0.001	1.691	0.976-2.930	0.061
Pathological grade (G3-G4 vs. G1-G2)	21.723	<0.001	2.181	1.268-3.752	0.005
T stage (T3-T4 vs. T1-T2)	22.563	<0.001	2.698	1.513-4.812	0.001
N stage (N2-N3 vs. N0-N1)	16.607	<0.001	2.016	1.201-3.384	0.008

HR: hazards ratio; *CI*: confidence interval.

下面积为0.764(95%*CI*：0.674~0.854, *P*<0.001, 图3)。取PCMT1相对表达量2.96为截点值, 敏感度为78.85%, 特异度为73.53%。

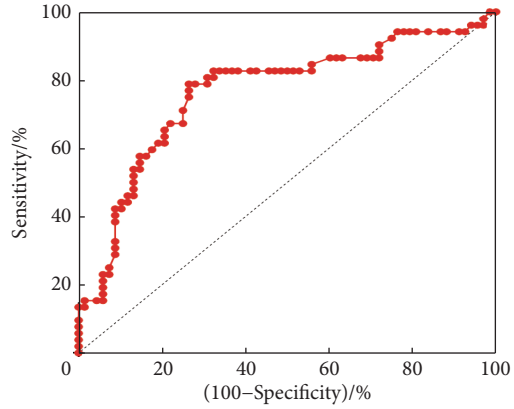

图 3 PCMT1表达预测胃癌患者术后5年生存率的ROC曲线

Fig 3 ROC curve analysis of PCMT1 expression to predict the 5-year survival of gastric cancer patients after radical gastrectomy

2.7 PCMT1促进胃癌MGC-803与HGC-27细胞的增殖

见图4。在两个细胞系中, 相比于sh-NC组, sh-*PCMT1*组PCMT1蛋白表达降低; 而与LV-Vec组相比, LV-*PCMT1*组PCMT1蛋白表达升高(*P*<0.05), CCK-8结果显示, 与sh-NC组相比, sh-*PCMT1*组MGC-803与HGC-27细胞的增殖能力下降, 而与LV-Vec组相比, LV-*PCMT1*组促进胃癌细胞的增殖能力(*P*<0.05)。

2.8 PCMT1抑制纺锤体组装检查点的激活并促进G_2/M期进程

见图5。在两个细胞系中, 与sh-NC组相比, sh-*PCMT1*组纺锤体检查点关键蛋白CDC20表达下调, CyclinB1表达升高; 与LV-Vec组相比, LV-*PCMT1*组CDC20表达上升而CyclinB1表达下降(*P*<0.05)。此外, sh-*PCMT1*组表达细胞周期阻滞于G_2/M期, LV-*PCMT1*组细胞周期G_2/M期阻滞作用减弱(*P*<0.05)。

2.9 PCMT1促进裸鼠皮下成瘤能力并抑制纺锤体组装检查点的激活

裸鼠皮下成瘤实验结果示, 与sh-NC组相比, sh-*PCMT1*组裸鼠移植瘤的体积与质量均减小, 过表达PCMT1肿瘤体积与质量较对照组增大(*P*<0.05, 图6A-6C)。免疫印迹结果进一步显示, 与sh-NC组相比, sh-*PCMT1*组的裸鼠肿瘤组织中CDC20蛋白表达降低, CyclinB1蛋白表达升高, 而与LV-Vec组相比, LV-*PCMT1*组的裸鼠肿瘤组织中CDC20表达上调, CyclinB1表达下调(*P*<0.05, 图6D)。

3 讨论

当今以手术切除为主的治疗方案并不能显著改善胃癌患者预后, 因此亟须开发新兴的治疗靶点以提高患者预后能力[11-12]。近年人们对分子靶点的研究逐渐成为探讨癌症发生发展及发病机制的主要方向[13-16]。PCMT1是一种蛋白质羧甲基转移酶, 该基因编码的酶在蛋白质修复中发挥重要作用。既往研究发现, PCMT1在乳腺癌组织中高表达且与患者不良预后相关[17]。然而尚未见PCMT1对胃癌作用的相关报道。因此, 本研究尝试分析PCMT1在胃癌中的表达及预后情况, 分析其潜在的分子机制, 为胃癌的诊疗革新思路。本研究假设PCMT1在胃癌组织中高表达, 可能影响胃癌患者预后, 可能通过调控有丝

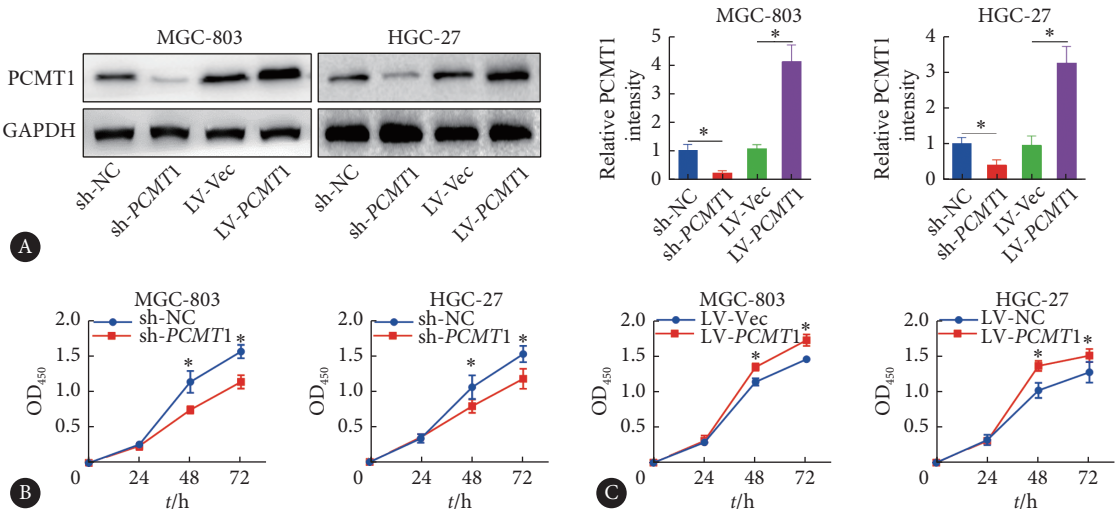

图 4 PCMT1对胃癌细胞增殖能力的影响

Fig 4 Effect of PCMT1 on the proliferation capacity of gastric cancer cells

A, Western blot assay (*n*=3); B, CCK-8 assay was done to determine the effect of interference with PCMT1 on the proliferation capacity of gastric cancer cells (*n*=5); C, CCK-8 was performed to determine the proliferation capacity of gastric cancer cells overexpressing PCMT1 (*n*=5). * *P*<0.05, vs. sh-NC or LV-Vec.

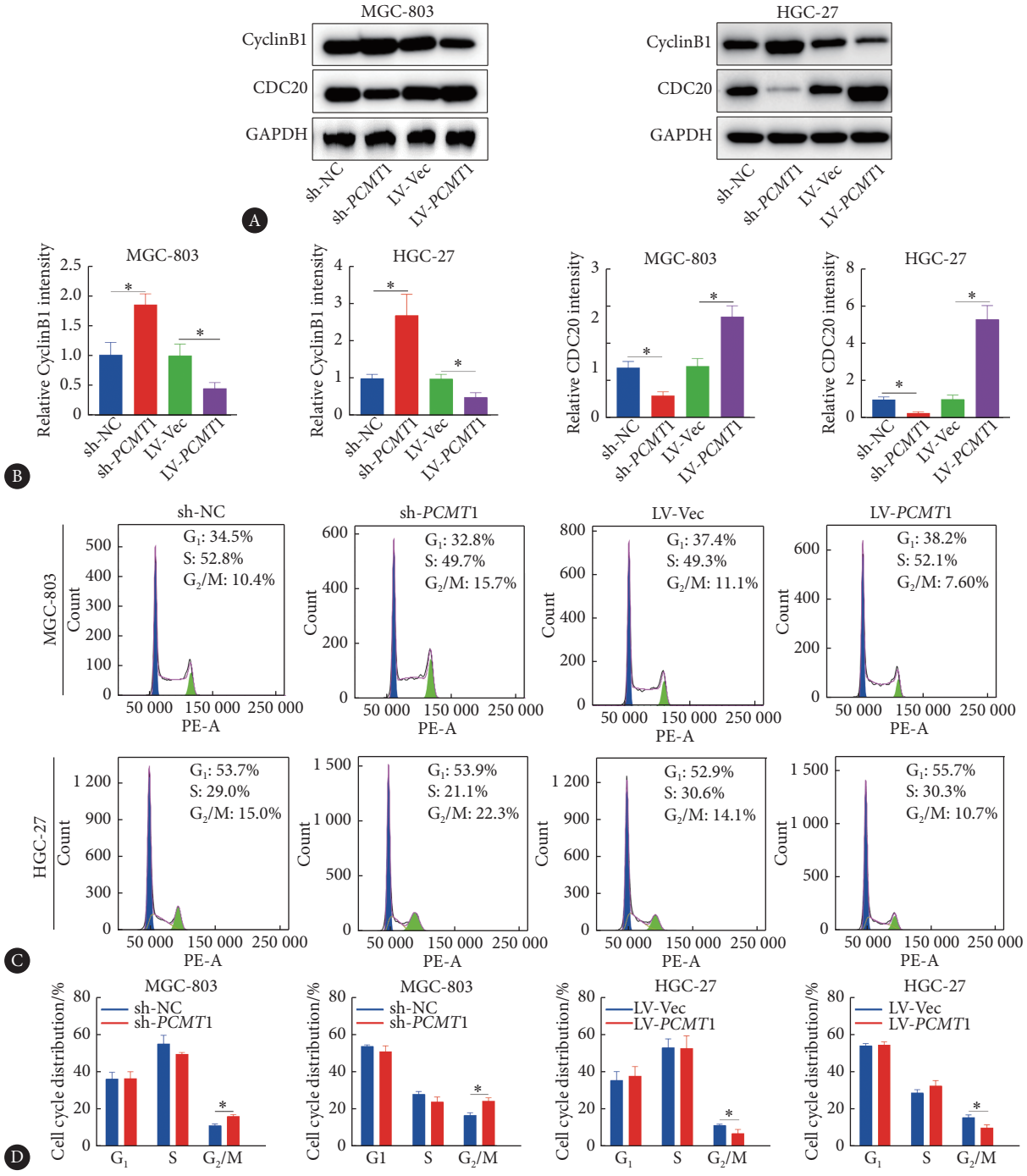

图 5　PCMT1对纺锤体组装检查点与细胞周期的调控

Fig 5　Regulation of spindle assembly checkpoints and cell cycle by PCMT1

A, Western blot results of the expression levels of key proteins, such as CyclinB1 and CDC20, that intervene in PCMT1 spindle assembly checkpoints; B, relative expression of CyclinB1 and CDC20 proteins; C, flow cytometry to determine the effect of PCMT1 intervention on cell cycle levels; D, statistical analysis of cell cycle distribution ratio. n=3, $^{*}P$<0.05.

分裂进程中SAC促进胃癌细胞的恶性增殖。本研究通过纳入胃癌患者临床病理参数，单因素与多因素分析PCMT1对胃癌患者术后5年生存率的影响，通过体内外实验进一步探讨PCMT1对胃癌细胞恶性增殖的影响及调控机制。

本研究通过UALCAN在线数据库与免疫组织化学法证实PCMT1在胃癌组织中呈高表达，且与癌细胞增殖标志物Ki67呈正相关，提示其可能参与胃癌的恶性进展。临床病理参数及生存分析进一步揭示高表达PCMT1与胃癌进展相关，且患者术后5年生存率明显缩短，提示预后不良。

此外，既往研究表明，PCMT1促进乳腺癌细胞的增

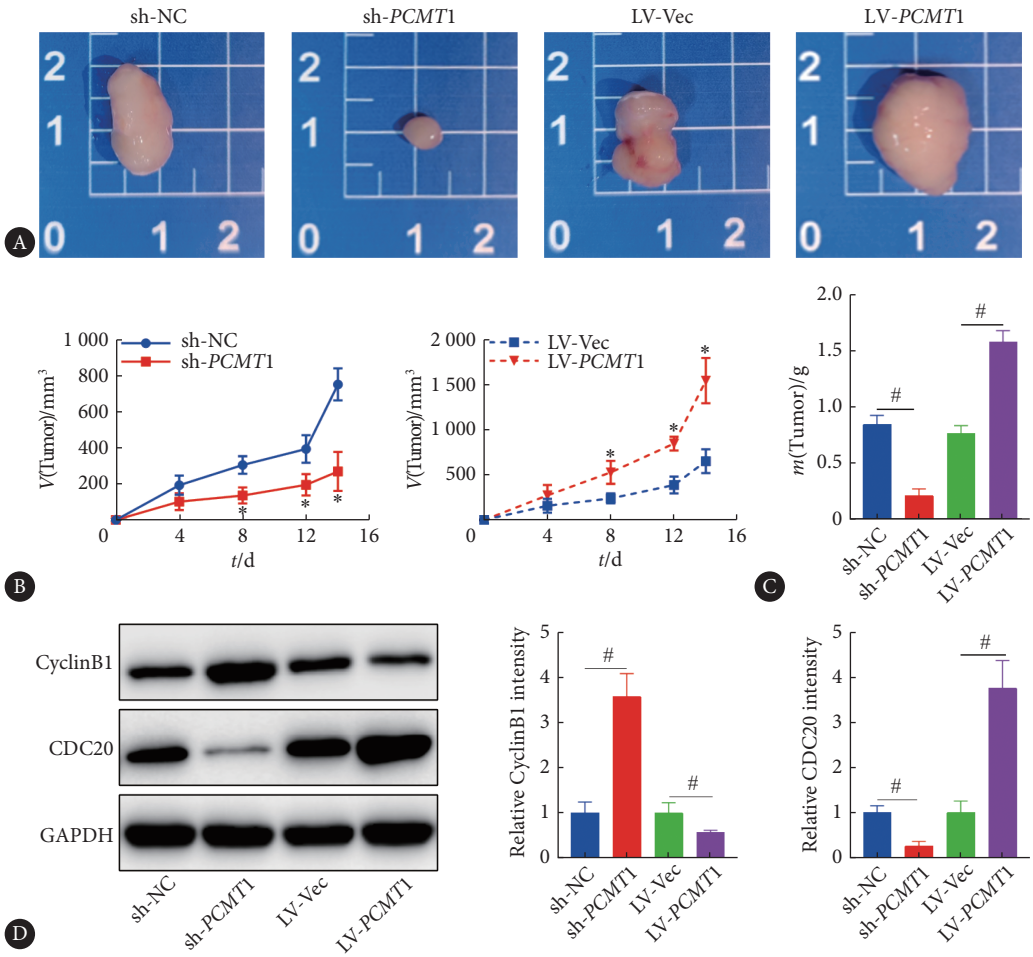

图 6 PCMT1对裸鼠皮下成瘤能力及纺锤体组装检查点的影响

Fig 6 Effect of PCMT1 on subcutaneous tumorigenesis and spindle assembly checkpoints in nude mice

A, Representative pictures of the tumors in nude mice; B, volume of the tumors; C, mass of the tumors; D, protein levels of CyclinB1 and CDC20 in the tumor xenograft tissues measured by Western blot assay. n=3, $^{*}P$<0.05, vs. sh-NC or LV-Vec; $^{\#}P$<0.05.

殖能力[18]。本研究通过体内外实验发现过表达PCMT1可促进胃癌细胞的增殖能力和裸鼠肿瘤的生长，干扰PCMT1则呈现相反趋势，提示PCMT1可能参与胃癌细胞的恶性增殖，然而，PCMT1促进胃癌细胞增殖的分子机制仍需进一步探索。

SAC负责有丝分裂染色体的准确分离，对调控细胞周期至关重要。SAC缺陷产生非整倍体，促进肿瘤的发生发展[19-20]。在肝癌细胞中敲除人类驱动蛋白家族成员4A(kinesin family member 4A, KIF4A)激活SAC，导致细胞周期阻滞于G_2/M期并促进多核细胞的形成，过表达KIF4A则引起细胞周期进展，促进肿瘤细胞的生长[21]。芹菜素通过调控CyclinB1的表达诱导胃癌细胞周期阻滞于G_2/M期，促进肿瘤细胞凋亡，发挥潜在的抗癌活性[22]。本研究通过GO与KEGG富集分析发现，PCMT1参与CDC20介导的有丝分裂、SAC、细胞周期等信号。体外实验证明干扰PCMT1的表达细胞周期阻滞于G_2/M期，且体内外实验均表明干扰PCMT1的表达纺锤体检查点关键蛋白CDC20表达下降、CyclinB1表达升高，过表达则呈相反趋势，提示PCMT1促进胃癌细胞异常增殖可能是通过抑制细胞有丝分裂进程中纺锤体检查点来实现。

本文的研究价值在于，本研究率先报道PCMT1在胃癌组织中呈高表达，且与肿瘤进展及患者不良预后相关。另外，本研究证实PCMT1可通过调控纺锤体检查点影响胃癌细胞的恶性增殖，该发现为临床上胃癌的治疗与评估预后提供更有价值的新型分子标志物。尽管如此，由于样本量有限，仍需进一步扩大样本量以提高临床数据的可信度。此外，PCMT1是否通过其他功能学途径及分子机制影响胃癌的发生发展仍有待进一步探讨。因此，本课题组下一步将对PCMT1调控胃癌细胞恶性增殖的作用及其他分子机制进行深入研究。

* * *

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研究方法, 张震负责数据审编, 李博涵负责软件, 黄菊负责调查研究, 李静负责经费获取, 耿志军负责验证, 张小凤负责可视化, 宋雪负责研究项目管理, 王炼负责提供资源, 左芦根负责经费获取和审读与编辑写作, 胡建国负责经费获取、监督指导和审读与编辑写作。所有作者已经同意将文章提交给本刊, 且对将要发表的版本进行最终定稿, 并同意对工作的所有方面负责。

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