MYBL2在前列腺癌患者组织中高表达并与不良预后相关

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摘要:目的 探索骨髓母细胞增生症病毒癌基因同源物样2(MYBL2)基因对前列腺癌(PCa)患者临床预后和生物学行为的影响。**方法** 采用qRT-PCR检测MYBL2在45例PCa与癌旁前列腺组织的表达水平,根据MYBL2表达中位数,分为高(23例)低(22例)表达,采用非参数检验、Kaplan-Meier、单因素和多因素Cox法,并分析MYBL2高低表达与PCa的临床病理特征及预后相关性,利用癌症基因组图谱(TCGA)基因芯片数据库PCa数据集进行验证。基因集富集分析(GSEA)MYBL2高低表达可能参与调控的分子通路,CIBERSORT算法研究MYBL2与肿瘤免疫微环境的相关性。最后,体内实验中,通过建立阴性对照组(shCtrl)、敲减MYBL2组(sh-MYBL2)用细胞增殖毒性检测试剂盒(CCK8)和Transwell法检测各组细胞增殖和侵袭能力。qRT-PCR和Western blotting检测肿瘤细胞中MYBL2基因和蛋白的表达。在体内实验中,检测2组荷瘤小鼠瘤重和免疫组织化学方法观察荷瘤组织Ki-67的表达水平。**结果** 两组数据均显示:MYBL2在PCa组织中高表达,与Gleason评分、临床和病理分期正相关(P<0.01),与年龄无相关性;Kaplan-Meier分析MYBL2高表达与无生化复发生存期显著负相关(P<0.05),与总生存期无关。Cox回归分析:TCGA数据集中临床和病理分期,我们的数据中临床分期和Gleason评分是PCa无复发生存期的独立预后因素(P<0.05)。GSEA结果显示高表达MYBL2与免疫、细胞粘附、细胞因子等通路有关。CIBERSORT分析:MYBL2表达与B cells memory和Mast cells resting有关(P<0.05)。体外研究显示,与shCtrl组相比,shMYBL2组能显著抑制LNCaP、PC-3细胞增殖和侵袭(P<0.01)。体内研究显示,shMYBL2组PC-3荷瘤小鼠体内肿瘤的平均重和Ki67阳性表达率显著低于shCtrl组(P<0.01)。**结论** MYBL2是一种致癌基因,与PCa多个病理指标相关,可以作为潜在的诊疗PCa患者预后的标志物和治疗肿瘤的靶标。
关键词:MYBL2;前列腺癌;细胞增殖;细胞侵袭;荷瘤小鼠;预后

High expression of MYBL2 promotes progression and predicts a poor survival outcome of prostate cancer

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Abstract: Objective To explore the correlation of MYB proto-oncogene like 2 (MYBL2) with biological behaviors and clinical prognosis of prostate cancer (PCa). **Methods** We detected Mybl2 mRNA expression in 45 pairs of PCa and adjacent tissues using real-time quantitative PCR, and analyzed the correlation of high (23 cases) and low expression (22 cases) of *Mybl2* with clinicopathological features and prognosis of the patients using nonparametric test, Kaplan-Meier survival analysis and univariate and multivariate Cox regression. The results were verified by analysis of the data from Cancer Genome Atlas (TCGA) microarray database, and the molecular pathways were identified by gene set enrichment analysis (GSEA). The CIBERPORT algorithm was used to identify the correlations between *Mybl2* expression and tumor microenvironment of PCa. We also tested the effects of MYBL2 knockdown on proliferation and invasion of PCa cell lines using cell counting kit-8 and Transwell assays and observed the growth of PC3 cell xenograft with MYBL2 knockdown in nude mice and the expression levels of Ki-67 in the xenograft using immunohistochemistry. **Results** *Mybl2* expression was significantly elevated in PCa tissues in close correlation with Gleason score and clinical and pathological stage of the tumor (P<0.01) but not with the patients' age. Kaplan-Meier analysis indicated a significant negative correlation of high *Mybl2* expression with recurrence-free survival (P<0.05), but not with the overall survival of the patients. The data from TCGA suggested that clinical and pathological stages were independent prognostic factors for recurrence-free survival, and our data indicated that clinical stage and Gleason score were independent prognostic factors of PCa (P<0.05). GSEA suggested that *Mybl2* expression was related with the pathways involving immune function, cell adhesion, and cytokine secretion; CIBERPORT analysis suggested the involvement of *Mybl2* expression with memory B cells and resting mast cells (P<0.05). In LNCaP and PC-3 cells, MYBL2 knockdown significantly inhibited cell proliferation and invasion (P<0.05); in the tumor-bearing nude mice, the xenografts derived from PC-3 cells with MYBL2 knockdown exhibited a lowered mean tumor weight and positivity rate for Ki67 (P<0.05). **Conclusion** *Mybl2* is an oncogene related with multiple pathological indicators of PCa and can serve as a potential prognostic marker as well as a therapeutic target for patients with PCa.
Keywords: *Mybl2*; prostate cancer; cell proliferation; cell invasion; tumor-bearing mice; prognosis

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前列腺癌(PCa)是男性泌尿生殖系统最常见的恶性肿瘤之一,PCa发病率和死亡率分别位居欧美男性恶性肿瘤的第1位和第3位,位居中国第6位和第7位,而在全球范围内其位居男性恶性肿瘤发病和死亡率的第2位和第5位[1-3]。世界范围内,近年来PCa的发病率一直呈现逐年增长的趋势,它是导致男性死亡的重要原因之一[4-5]。近年来,前列腺特异性抗原常规应用临床诊疗中,已经取得了很好的成效,其对PCa的早期诊断和预后评估有很大帮助[6-7]。但是,由于PCa是高度异质性疾病,疾病结局不尽相同,临床上尽早对PCa患者进行风险和预后评估显得尤其重要[8-9]。因此,迫切需要探索与PCa发生发展预后相关的基因,为PCa的早期诊断和个体化治疗提供理论依据。

骨髓母细胞增生症病毒癌基因同源物样2(MYBL2)基因又名B-myb,是MYB家族成员之一,在多种肿瘤细胞中广泛表达[10-11]。MYBL2参与调节细胞增殖、凋亡周期和细胞周期等多种生理病理过程,因此被认为参与肿瘤的发生发展[12-14]。MYBL2与肺癌、肝癌、乳腺癌、胃癌等的发生发展以及预后有关[15-18]。MYBL2还与卵巢癌耐药性有关,在卵巢癌细胞系SKOV3中,MYBL2可以通过破坏细胞周期而促进卵巢癌细胞的侵袭[19-21]。但是,关于MYBL2在前列腺癌方面的研究较少,其中,研究发现MYBL2过表达干扰Hippo-YAP信号通路,促进PCa去势抵抗和转移[22]。通过生物信息数据库中数据研究发现MYBL2在PCa组织中显著上调表达,并与患者不良临床病理特征相关[23]。但是关于MYBL2生物信息分析与临床结合基础研究尚未见报道。因此进一步探索研究MYBL2表达与PCa的相关性十分必要。

本研究通过分析癌症基因组图谱(TCGA)数据库和我们收集的临床数据中的MYBL2基因表达及临床病理特征数据,分析MYBL2与临床预后关系。通过CIBERSORT算法和GSEA基因集富集分析方法分析MYBL2高低表达调控的相关通路和免疫细胞,最后通过体外、体内实验来探索MYBL2在前列腺癌中的潜在作用。从而探讨PCa中MYBL2表达及临床预后意义,将为PCa的防治提供理论依据。

1 资料和方法

1.1 材料

1.1.1 *标本来源* 收集2010年6月~2012年6月南京中医药大学附属医院和南京中医药大学第二附属医院PCa组织(表1)及其相应癌旁正常组织各45例,严格遵守此次研究的纳入、排除标准。纳入标准:首次确诊并行PCa根治术,术前均未行放射治疗或化学治疗;患者知情同意参加本次研究。排除标准:合并其他恶性肿瘤;合并其他影响MYBL2表达的特异性疾病。本研究患者随访中位时间为69.4个月,平均随访时间 73.2±19.2个月,术后5年内3个月随访,以后每半年随访1次。所有入组患者均征得其同意并签署知情同意书,本研究征得医院伦理委员会同意批准实施。验证数据集:通过癌症基因组图谱(TCGA)基因芯片数据库下载合并MYBL2 mRNA表达与PCa临床特征数据。其中包括481例PCa(表2)及51例非癌组织。

表1 临床数据信息表

Tab.1 Clinical information of the 45 patients with PCa

Varialbe	Classification	Number of tumor samples (*n*)
Age(year)	<65	12
	≥65	33
Gleason score	<8	27
	≥8	18
Clinical stage	<T3	31
	≥T3	14
Pathologic stage	<T3	27
	≥T3	18
MYBL2 expression	Low	22
	High	23

表2 TCGA数据库的临床信息表

Tab.2 Patient data from TCGA database

Project	Classification	Number of tumor samples (*n*)
Age(year)	<65	319
	≥65	162
Gleason score	<8	285
	≥8	196
Clinical_S	<T3	344
	≥T3	53
Pathologic_S	<T3	186
	≥T3	288
MYBL2	low	241
	high	240

1.1.2 *细胞和动物* 细胞系:LNCaP、PC-3、RWPE-1购于武汉普诺赛生命科技有限公司。动物:SPF级雄性裸鼠(年龄6~8周,体质量16~18 g)受赠于南京中医药大学动物实验室并通过伦理审查。动物许可证号:SYXK(苏)2018-0049。

1.1.3 实验试剂 细胞培养基(DMEM)及0.25%胰蛋白酶(HyClone);胎牛血清(FBS)(Gibco);siRNA转染试剂盒(Thermo Fisher);CCK-8(CK04)试剂盒(江苏,凯基);mRNA逆转录试剂盒和SYBR Green试剂盒(Takara);β-actin抗体(1∶2000,Abcam);MYBL2抗体(1∶1000,Abcam);兔源二抗(1∶2000,CW0102M,CWBIO);小鼠源二抗(1∶4000,Biosharp);Ki-67抗体(1∶1000,Bioss Co. Ltd)。

1.2 方法

1.2.1 基因集富集分析(GSEA) 将TCGA数据集中的PCa样本根据MYBL2表达值的中位数分为高低组,利用GSEA 4.0.3软件进行基因富集分析,参数条件:置换次数1000次,P<0.05,FDR<0.25,最后得到MYBL2基因的表达水平与调控基因富集通路的关系。

1.2.2 CIBERSORT算法分析 提取TCGA数据集中PCa组织及正常组织转录本中的MYBL2 mRNA表达谱数据,使用R语言limma包对数据进行校正,Cibersort包"反卷积法"得到22种免疫细胞基准数据库文件即各种细胞占比。根据P<0.05筛选样本,按照MYBL2表达值的中位数分为高低组状态统计绘制相关图。

1.2.3 细胞培养和转染 10%胎牛血清,1%青、链霉素和DMEM培养基进行培养,培养箱温度为37 ℃、CO_2浓度5%,保持一定湿度。当LNCaP、PC-3细胞融合度至50%以上时,使用Lipofectamine2000对细胞进行转染,操作步骤按说明书进行。转染成功后培养24~72 h后进行后续实验。

1.2.4 CCK8实验 取对数生长期的LNCaP、PC-3细胞,将细胞(2×10^3/孔)置于96孔板中,并在培养后24、48、72 h的3个时间段通过CCK-8试剂盒检测,操作步骤按说明书进行。

1.2.5 Transwell实验 用无血清培养基将细胞稀释至细胞密度4×10^5/mL,加入Transwell小室的上室进行包被,取200 μL细胞悬液加入小室,600 μL 10%胎牛血清的培养液加入下室,37 ℃培养箱中培养24~48 h,随后多聚甲醛固定,结晶紫染色,最后拍照并计数统计。

1.2.6 蛋白质免疫印迹实验 提取总蛋白后,BCA蛋白质检测试剂盒进行蛋白定量。30 μg蛋白样品加载到配制好的SDS-PAGE凝胶上分离,后转印到聚偏氟乙烯(PVDF)膜上封闭2 h,洗涤膜后加入相应一抗,在4 ℃下孵育过夜。洗涤膜后加入相应二抗,25 ℃下孵育1.5 h,洗涤膜后显影、曝光,以β-actin进行标定,用Image J软件分析目的蛋白表达水平。

1.2.7 实时定量PCR实验 TR-Izol试剂盒提取总RNA,PrimeScript RT-PCR试剂盒用于逆转录,SYBR Green PCR试剂用于使用Bio-Rad-PCR仪器进行RT-PCR。引物如下:MYBL2: forward: CTTGA-GCGAGTCCAAAGACTG, reverse: AGTTGGTCAG AAGACTTCCC; β-actin: forward: GTTG-CTATCCA GGCTGTGCTATCC, reverse: CTGTGAGTCAGG-GT CCACAC。数据采用$RQ=2^{-\Delta\Delta Ct}$法分析MYBL2 mRNA的表达水平。

1.2.8 造模 取对数生长期且转染成功后的PC-3细胞,然后将细胞活力为95%以上的细胞悬液接种于裸鼠的背部皮下,接种0.1 mL/只,成瘤标准为背部皮下瘤体直径达0.5 cm。所有实验操作均按照美国国立卫生研究院《实验动物使用指南》的相关规定进行。实验方案经南京中医药大学动物伦理委员会审核通过。

1.2.9 称量瘤重和免疫组化染色分析 干预结束后第2天,颈椎脱臼法处死小鼠,取出完皮下肿瘤,用天平称量瘤重。瘤体标本石蜡包埋后切片,免疫组化操作按试剂盒要求,二氨基联苯胺显色,苏木精复染,二甲苯封片。Ki67阳性定位以组织切片中胞核染色为棕黄色颗粒。阳性率=阳性目标面密度/观察目标面密度。

1.3 统计学分析

数据用R语言,Graphpad5.0软件进行统计分析和作图。计量资料结果用均数±标准差表示,先进行方差齐性检验,再行非参数检验或t检验,分析MYBL2高低表达与临床资料的相关性。Kaplan-Meier、Cox回归分析与临床预后的相关性。P<0.05时认为差异有统计学意义。

2 结果

2.1 MYBL2在PCa和癌旁组织中的表达情况

通过对45例PCa患者肿瘤组织与癌旁正常组织中MYBL2的mRNA相对表达水平进行比较,发现MYBL2在PCa组织呈高表达(图1A)。并在TCGA基因芯片数据库中PCa数据集得到验证(481例PCa肿瘤组织与51癌旁正常组织)(图1B)。

2.2 MYBL2的表达与PCa临床病理因素的关系

进一步对PCa组织中MYBL2的表达情况与患者临床病理因素之间的关系进行分析,发现MYBL2的高表达与Gleason score、临床和病理分期密切相关,与年龄无关(图2A~D),TCGA数据集分析亦发现相同结果(图2E~H)。

2.3 MYBL2的表达与PCa预后的关系

经log-rank检验MYBL2高表达与无生化复发生存率具有显著负相关性(图3A),与总生存期无关(图3B)。COX比例风险模型结果表明Gleason score(HR=3.56, 95% CI:1.03- 12.38, P=0.045)及临床分期(HR=8.43,95% CI: 2.20-32.31,P=0.002)是PCa患者生化复发的独立预后影响因素(表3)。TCGA数据集分析:MYBL2高表达与无生化复发生存率具有显著负相

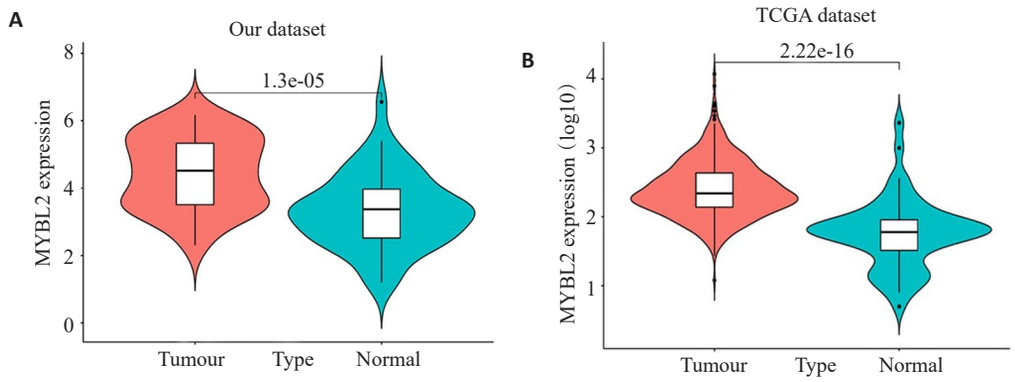

图1 MYBL2在PCa组织中高表达

Fig.1 MYBL2 is highly expressed in PCa tissues. **A**: Violin diagram of the relative expression of MYBL2 in tumor and adjacent tissues in the 45 PCa patients. **B**: Violin diagram of the relative expression of MYBL2 in tumor and non-tumor specimens in TCGA dataset.

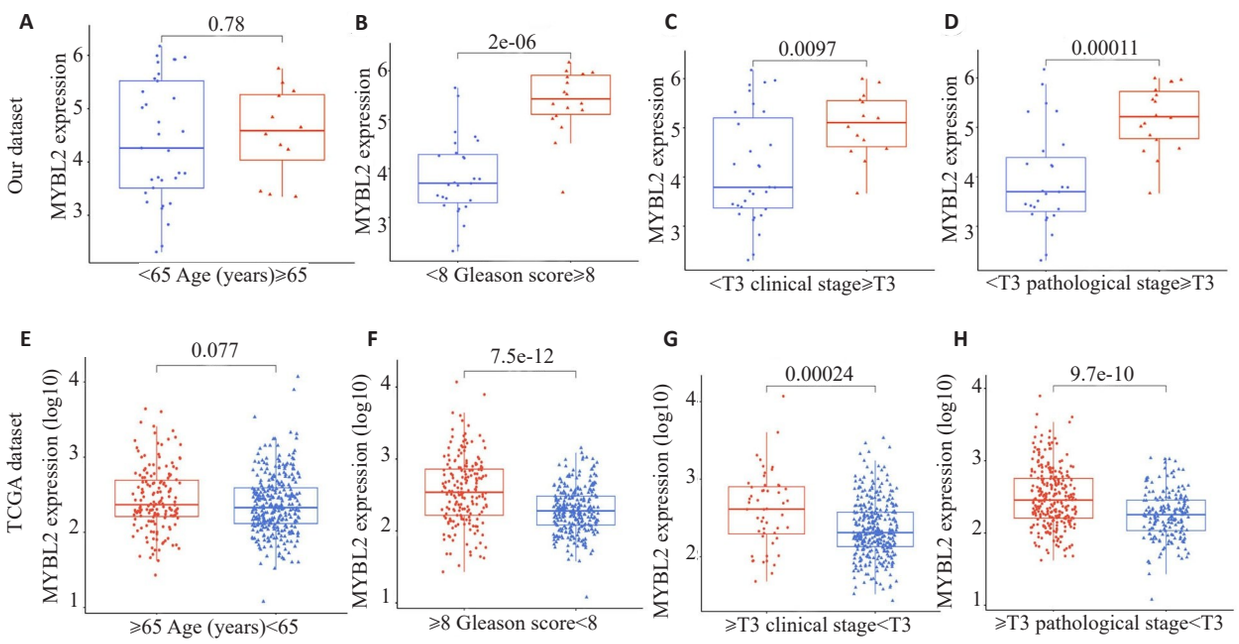

图2 MYBL2表达与PCa临床病理因素的相关性

Fig.2 Correlation between MYBL2 expression and clinicopathological factors of PCa. **A-D**: Box plots of the correlation of MYBL2 expression with clinicopathological features of the 45 PCa patients. **E-H**: Box plots of the correlation of MYBL2 expression with the clinicopathological factors in cases from TCGA dataset.

关性(图3C),与总生存期无关(图3D)。COX比例风险模型结果表明临床分期(HR=2.2,95% *CI*: 1.2-4.2,*P*=0.012)和病理分期(HR=2.6,95% *CI*: 1.0-6.5,*P*=0.047)是PCa患者生化复发的独立预后影响因素(表4)。

2.4 MYBL2高低表达的GSEA分析

标准化处理TCGA数据库中的前列腺癌全转录组数据,将MYBL2高、低两组中的其他转录组基因进行了GSEA富集分析,发现与其正相关的通路主要包括免疫、细胞粘附、细胞因子等通路,与其负相关的通路主要有蛋白质代谢、细胞周期等通路(图4)。

2.5 MYBL2高低表达的PCa免疫微环境

利用CIBERSORT算法观察22种肿瘤免疫细胞在PCa组织中的聚类情况(图5A),在MYBL2高表达和低表达组中上述肿瘤免疫细胞比例和亚群分布有显著性差异(图5B~D)。此外,在TCGA数据集中B cells memory与MYBL2表达呈正相关,mast cells resting与MYBL2表达呈负相关(图5E~F)。

2.6 MYBL2在细胞中的表达及转染效果的验证

采用qRT-PCR和Western blotting法检测MYBL2在人PCa细胞LNCaP、PC-3细胞和人正常前列腺上皮细胞RWPE-1中的表达。结果表明,MYBL2在LNCaP、PC-3细胞中高表达,在RWPE-1低表达(图6A,*P*<0.001)。LNCaP、PC-3细胞中,shMYBL2组MYBL2的表达明显低于shCtrl组(图6B,*P*<0.001)。

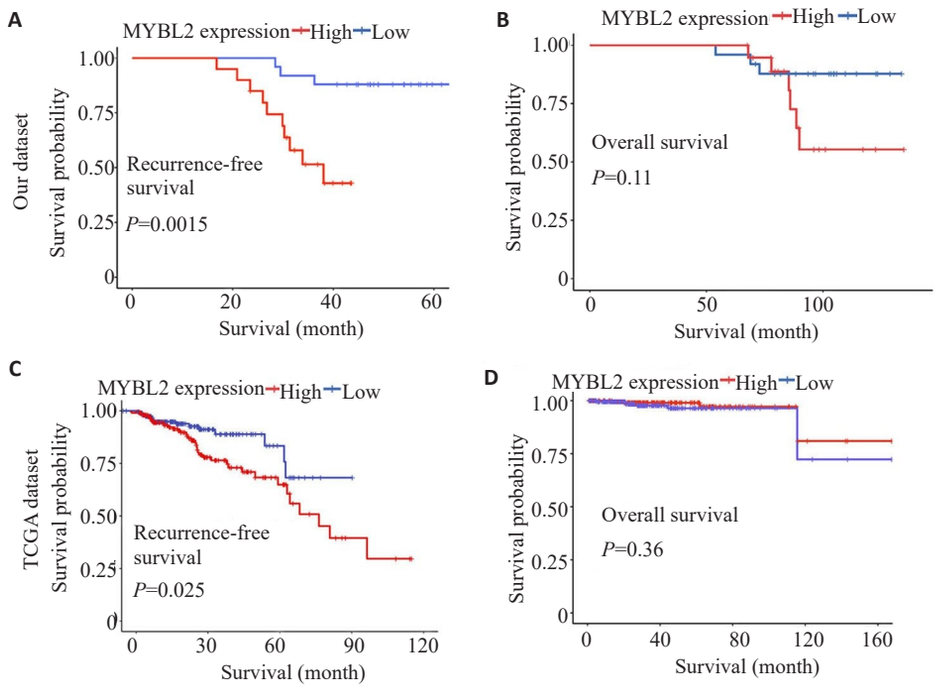

图3 MYBL2表达与PCa预后的相关性

Fig.3 Correlation between MYBL2 expression and prognosis of PCa patients. **A-B**: Kaplan-Meier analysis of high and low MYBL2 expression groups in our dataset; **C-D**: Kaplan-Meier analysis of high and low MYBL2 expression groups in our TCGA dataset.

表3 PCa生化复发的独立预后因素

Tab.3 Independent prognostic factors of biochemical recurrence in the 45 patients with PCa

Subgroup	Univariate_cox			Multivariate_cox		
	HR	95% *CI*	*P*	HR	95% *CI*	*P*
Age (year) (≥65,<65)	0.78	0.24-2.5	0.68	-	-	-
Clinical stage (<T3 *vs* ≥T3)	0.086	0.023-0.32	0.00023	8.43	2.20-32.31	0.002
Pathologic stage(<T3 *vs* ≥T3)	0.14	0.037-0.5	0.0025	-	-	-
Gleason score(<8 *vs* ≥8)	0.17	0.05-0.55	0.0035	3.56	1.03-12.38	0.045
MYBL2 (low *vs* high)	0.13	0.028-0.57	0.0073	-	-	-

表4 TCGA数据库中PCa生化复发的独立预后因素

Tab.4 Independent prognostic factors of biochemical recurrence of PCa in cases from TCGA database

Subgroup	Univariate_cox			Multivariate_cox		
	HR	95% *CI*	*P*	HR	95% *CI*	*P*
Age (year) (≥65, <65)	1.2	0.69-2.1	0.49	-	-	-
Clinical stage (<T3 *vs* ≥T3)	3.7	2.1-6.7	1.40E-05	2.2	1.2-4.2	0.012
Pathologic stage (<T3 *vs* ≥T3)	4.2	1.8-9.8	0.00099	2.6	1.0-6.5	0.047
Gleason score(<8 *vs* ≥8)	3.1	1.7-5.6	0.00034	-	-	-
MYBL2 (low *vs* high)	1.9	1.1-3.5	0.028	-	-	-

2.7 MYBL2促进前列腺癌细胞增殖和侵袭

CCK-8实验结果显示，与shCtrl组相比，shMYBL2组能够显著抑制LNCaP和PC-3细胞的增殖（图6C）；Transwell实验结果显示与shCtrl组相比，shMYBL2组能够显著抑制LNCaP和PC-3细胞的侵袭（图6D）。

2.8 MYBL2对小鼠肿瘤生长和Ki-67表达的影响

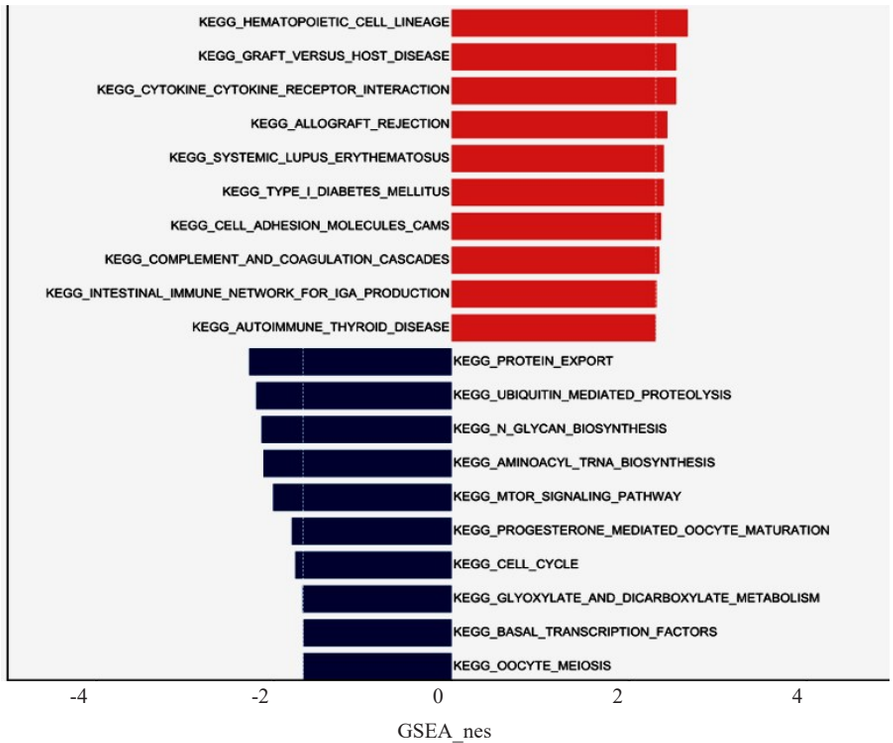

图4　MYBL2表达高低分组的GSEA富集分析

Fig.4　GSEA enrichment analysis of high and low MYBL2 expression groups.

shCtrl组和shMYBL2组瘤重分别为1.14±0.12 g、0.65±0.11 g，与shCtrl组相比，shMYBL2组显著抑制肿瘤的生长（图7A，$P<0.01$）；与shCtrl组相比，shMYBL2组MYBL2蛋白表达显著降低（图7B，$P<0.01$）；shMYBL2组和shCtrl组Ki-67阳性表达率分别为(39.70±4.21)%、(72.40±6.10)%，与shCtrl组相比，shMYBL2组Ki-67阳性表达率降低（图7C，$P<0.01$）。

3　讨论

MYBL2作为癌基因编码转录因子，在肿瘤细胞的细胞调控、增殖、分化和自噬中发挥重要作用，同时在一定条件下可以维持胚胎发育、组织稳态[24-25]。研究发现MYBL2的过表达与多种肿瘤发生发展密切相关[26-27]。MYBL2过表达促进肿瘤细胞增殖、转移，同时可以介导癌细胞耐药，从而参与癌症的发生、发展[28-29]。在多种癌症中p53突变与MYBL2过表达呈正相关，同时可以协同P53发挥促癌作用[30]。此外，在乳腺癌中MYBL2基因过表达，可下调E-cadherin表达，MYBL2基因低表达，能上调E-cadherin表达，结果提示MYBL2调控上皮－间质转化信号通路，促进细胞侵袭，而发挥促癌作用[31]。上述研究表明MYBL2在多种肿瘤发生发展中发挥着重要的作用，但关于MYBL2在PCa中的研究比较少。

本实验结果发现，PCa组织中MYBL2 mRNA的相对表达量高于癌旁正常前列腺组织，MYBL2在PCa患者中过表达，并与不良预后相关。本次所收集的临床数据研究表明，MYBL2蛋白高表达与Gleason score、临床病理特征相关，而与年龄无相关性。通过TCGA数据验证也得到同样结果。本组预后分析显示，临床分期和Gleason score同为PCa无生化复发生存率的独立预后因素。此外，利用TCGA数据库进一步证实，MYBL2蛋白表达与PCa患者无生化复发生存率呈负相关，但不是PCa无生化复发生存率的独立预后因素。本次研究结果与TCGA数据集验证结果存在一定的偏差，分析其可能由于本组病例总体样本量较少，故后期需要开展大样本多中心研究加以验证。

研究发现，MYBL2的高表达预示着肾癌预后不良，我们分析发现MYBL2的表达增强提示PCa患者预后不良[32]，这说明MYBL2的高表达可能与诸多肿瘤的不良预后有关。接下来，为了揭示MYBL2在PCa中的生物学作用，在体外研究中我们用慢病毒稳定转染LNCaP和PC-3细胞，下调MYBL2的表达，进而建立了shCtrl组和shMYBL2组，结果表明，MYBL2基因敲除后，PCa细胞的增殖和侵袭能力明显降低。在体内研究中我们用慢病毒稳定转染下调MYBL2的表达的PC-3细胞制作荷瘤模型，结果表明，MYBL2基因敲除后PCa细胞的平均重量显著下降。上述研究结果证实抑制MYBL2表达可发挥抗癌作用。具体作用机制有待进一步分析。

PCa免疫微环境的改变与前列腺癌发生、发展及预

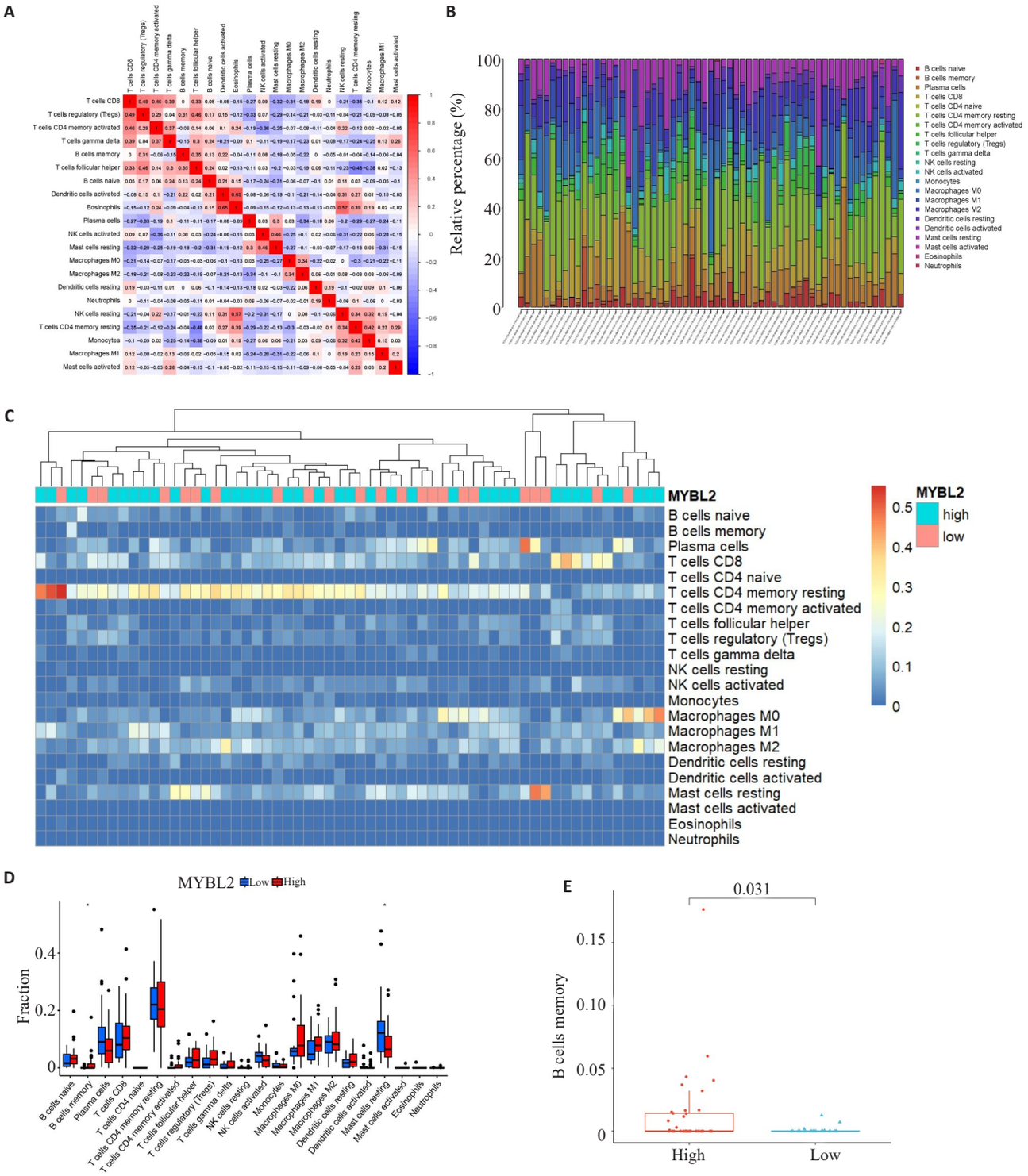

图5 MYBL2表达高低分组的免疫相关分析

Fig.5 Immunoassay results of high and low MYBL2 expression groups. **A**: Correlation matrix of 22 immune cells. **B**: Differences in the 22 immune cells between high and low MYBL2 expression groups. **C**: Heatmap of the 22 immune cells in high and low MYBL2 expression groups. **D**: Box plot of the differences in the distribution of 22 immune cells between high (red) and low (blue) MYBL2 expression groups. **E**, **F**: High and low MYBL2 expressions are correlated with memory B cells and resting mast cells.

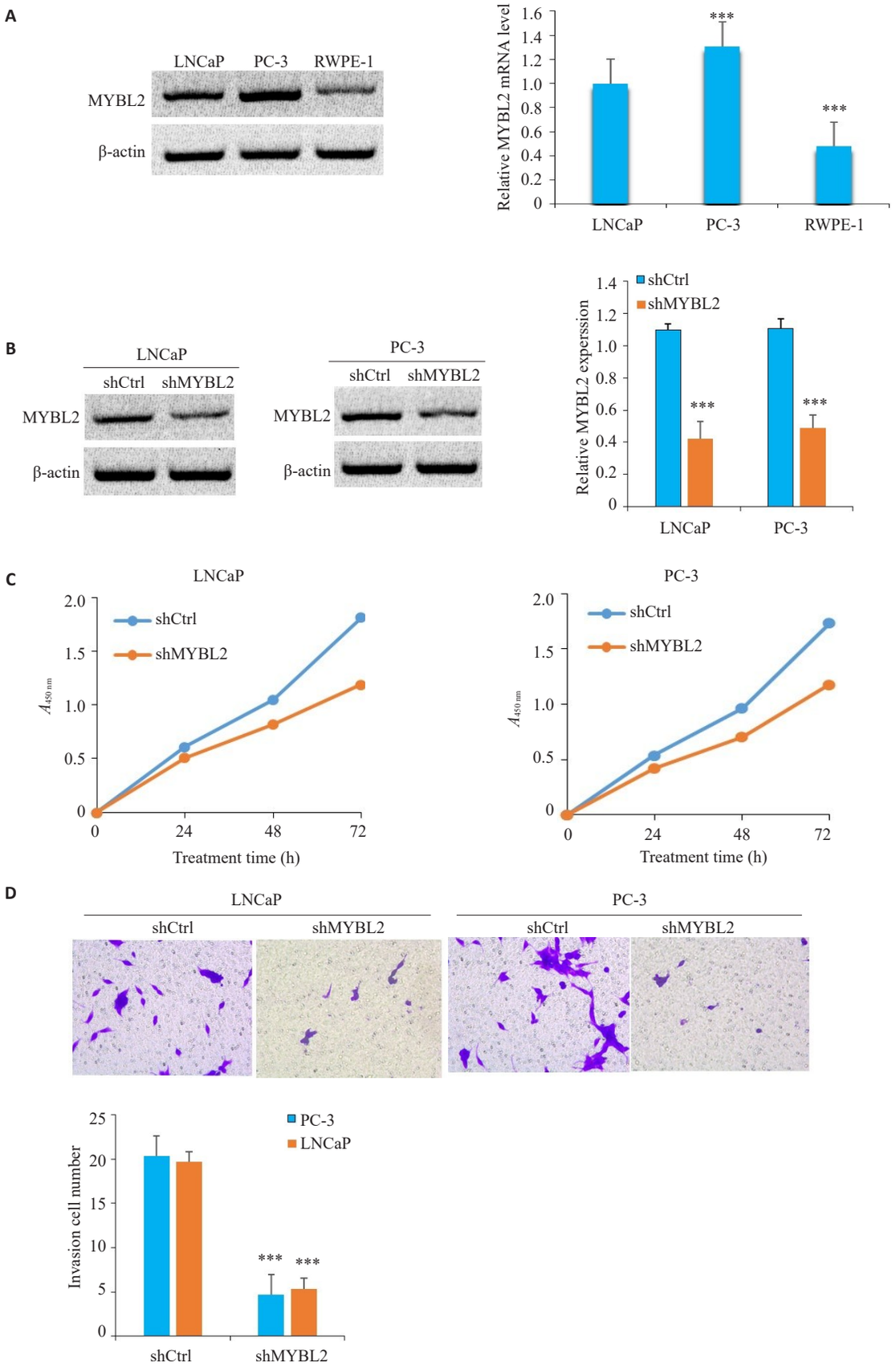

图6 MYBL2基因下调对PCa细胞增殖和侵袭的影响

Fig.6 Effects of MYBL2 knockdown on proliferation and invasion of PCa cells. **A**: MYBL2 expression in LNCaP and PC-3 cells and prostate hyperplasia (RWPE-1) cells. **B**: MYBL2 knockdown significantly inhibits MYBL2 expression in LNCAP and PC-3 cells. **C**: MYBL2 knockdown significantly inhibits proliferation of LNCaP and PC-3 cells. **D**: MYBL2 knockdown significantly inhibits invasion of LNCaP and PC-3 cells (× 400). **P*<0.01, ****P*<0.001 *vs* shCtrl group.

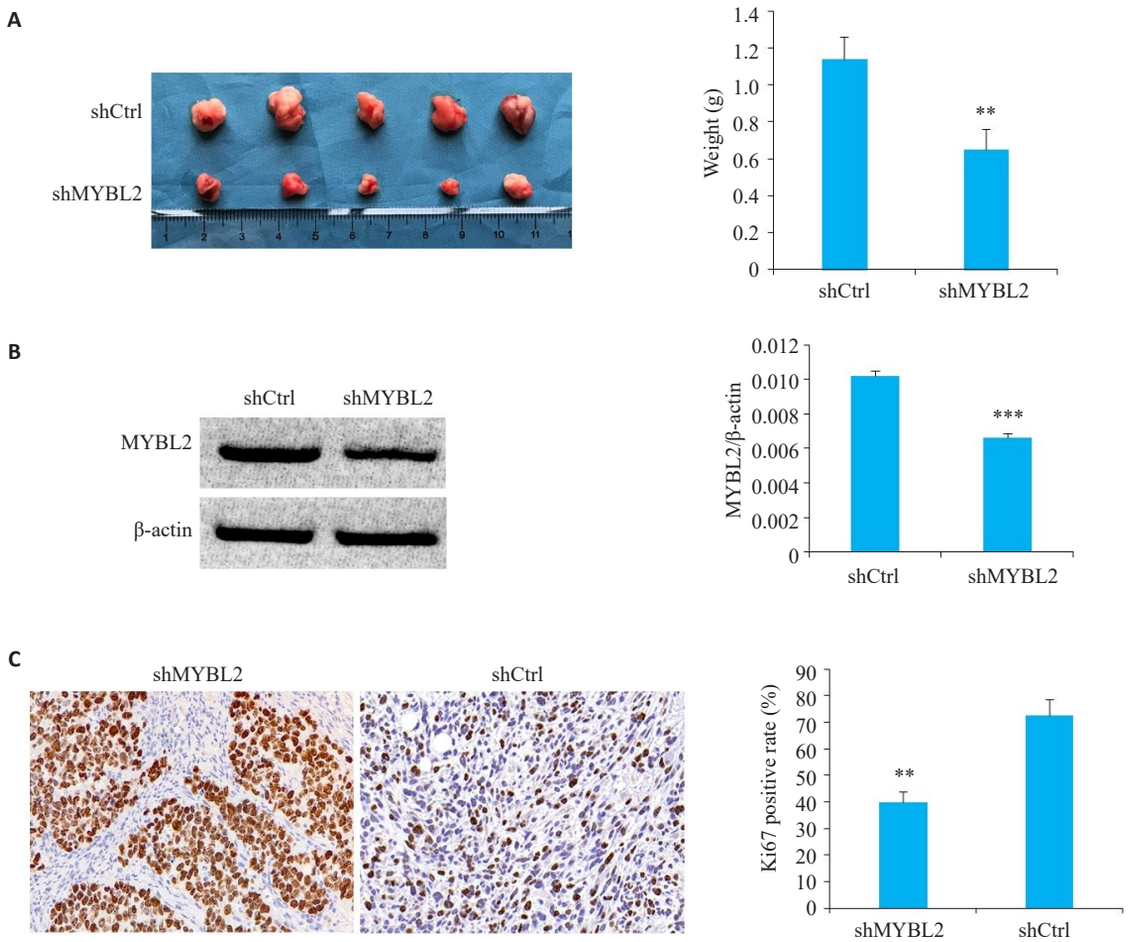

图7 MYBL2基因下调对PCa荷瘤小鼠的影响

Fig.7 Effects of MYBL2 knockdown on growth of PC-3 cell xenografts in nude mice. **A**: MYBL2 knockdown in PC-3 cells significantly inhibits tumor growth in nude mice. **B**: MYBL2 knockdown in PC-3 cells significantly inhibits the expression of MYBL2 in the xenograft in nude mice. **C:** MYBL2 knockdown in PC-3 cells significantly inhibits the expression of Ki-67 in the xenograft in tumor-bearing mice (×400). ***P*<0.01, ****P*<0.001 *vs* shCtrl group.

后结局密切相关[33-34]。本研究应用CIBERSORT评估了PCa及正常前列腺组织中浸润的免疫细胞比例，了解PCa组织中的22种免疫细胞浸润模式。通过GSEA基因富集分析发现MYBL2高低表达可参与调控前列腺免疫浸润。先前已有报道，肥大细胞和B细胞存在于PCa的免疫细胞浸润中，与PCa患者的恶性程度和转移密切相关[35-36]。本研究还发现，MYBL2高低表达与B cells memory和Mast cells resting的免疫浸润水平具有显著差异，提示MYBL2在免疫微环境中的作用是通过这两类免疫细胞浸润实现的。本研究观察到与文献报道相同的现象。我们推测MYBL2参与PCa免疫浸润调控，后续实验我们将进一步进行验证。

综上所述，本研究表明MYBL2在PCa中高表达，其与PCa的Gleason score、临床和病理分期密切相关，而且与PCa的不良预后显著相关。抑制MYBL2的表达可以抑制PCa细胞的增殖、侵袭能力和抑制荷瘤小鼠皮下肿瘤的生长，这为进一步研究MYBL2在PCa发生发展中可能的分子机制奠定了基础，并有望为PCa的靶向治疗提供新的参考和方向。

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