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INPP4B促进结直肠癌转移及其作用机制的初步探索*

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【摘要】 **目的** 明确肌醇多磷酸4-磷酸酶Ⅱ型（inositol polyphosphate-4-phosphatase type Ⅱ B, INPP4B）在结直肠癌（colorectal cancer, CRC）中的表达及临床意义，明确CRC细胞中INPP4B与基质金属蛋白酶7（matrix metallopeptidase 7, MMP7）的关系，并初步探究INPP4B对CRC细胞的增殖、迁移的影响及机制。**方法** 使用TIMER2.0和GEPIA2数据库分析*INPP4B*在癌（瘤）和癌旁（瘤旁）组织中的表达差异和对CRC预后的影响；通过免疫组化检测临床手术切除的102例CRC肿瘤中INPP4B的表达，并分析INPP4B与临床病理指标的相关性；在过表达/敲减*INPP4B*的CRC细胞中，实时荧光定量PCR检测*INPP4B*和*MMP7*的基因表达，Western blot检测INPP4B蛋白表达，利用CellTiter 96® AQueous One检测细胞增殖，划痕实验、实时无标记动态细胞分析技术（RTCA）检测细胞迁移和侵袭；结合LinkedOmics数据库分析与*INPP4B*功能相关信号通路，并在细胞水平验证潜在的关键分子。**结果** 数据库分析结果显示，与正常组织相比（结肠癌：1.91，直肠癌：1.89），在CRC中*INPP4B*表达升高（结肠癌：2.30，直肠癌：2.33）。免疫组化检测临床肿瘤组织和肿瘤旁组织也证实INPP4B在CRC中表达增高（$P<0.001$）。Cox回归模型分析显示*INPP4B*〔风险比（HR）=1.457，95%置信区间（CI）：1.003～2.115〕影响CRC预后，Kaplan-Meier曲线显示*INPP4B*高表达患者生存期短（$P<0.05$）；χ^2检验分析INPP4B表达与临床病理指标，发现INPP4B的高表达与淋巴结转移（$\chi^2=3.997$，$P=0.046$）和神经浸润（$\chi^2=8.511$，$P=0.004$）相关。体外实验中，与对照组细胞相比，过表达*INPP4B*的CRC细胞增殖和迁移能力增加（$P<0.05$）。通过LinkedOmics数据库分析显示*INPP4B*与细胞外基质重塑和细胞转移相关；Pearson相关性分析显示*MMP7*与*INPP4B*正相关（$r=0.378\,2$，$P<0.001$）；体外过表达或敲减*INPP4B*后*MMP7*表达水平也随之升高和下降。**结论** INPP4B在结直肠肿瘤组织中呈高表达并与淋巴结转移、神经浸润、患者预后相关。*MMP7*可能介导了INPP4B促进CRC细胞迁移和侵袭的作用。

【关键词】 肌醇多磷酸4-磷酸酶Ⅱ型 结直肠癌 基质金属蛋白酶7

Preliminary Study of the Role of INPP4B in Promoting Colorectal Cancer Metastasis and the Mechanisms Involved

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【**Abstract**】 **Objective** To investigate the expression of inositol polyphosphate 4-phosphatase type Ⅱ B (INPP4B) in colorectal cancer (CRC) and the relevant clinical significance, to determine the relationship between INPP4B and matrix metallopeptidase 7 (MMP7) in CRC cells, and to make preliminary exploration of the effects of INPP4B on the proliferation and migration of CRC cells and mechanisms involved. **Methods** The TIMER2.0 and GEPIA2 databases were used to analyze the differences in *INPP4B* expression between cancer and para-cancerous tissues and the effects of such differences on the prognosis of CRC. The expression of INPP4B in 102 surgically resected CRC tumors was determined by immunohistochemistry (IHC), and the correlation between INPP4B and clinical pathological indicators was analyzed. In CRC cells with overexpressed/knocked-down *INPP4B*, the expression of *INPP4B* and *MMP7* were examined by real time fluorogenic quantitative PCR, the protein expression of INPP4B was assessed by Western blot, cell proliferation was determined using the CellTiter 96® AQueous One assay, and cell migration and invasion were assessed using wound healing assay and real-time label-free dynamic cell analysis (RTCA). The LinkedOmics database was used to analyze signaling pathways related to *INPP4B* function, and the role of potential key molecules was validated at the cellular level. **Results** Analysis with the TIMER2.0 database and GEPIA2 database showed elevated *INPP4B* expression (colon adenocarcinoma [COAD]: 2.30, rectal adenocarcinoma [READ]: 2.33) in CRC compared to normal tissue (COAD: 1.91, READ: 1.89). IHC testing confirmed that INPP4B was upregulated in clinical CRC tissues and paracancerous tissues ($P<0.001$). Cox regression model analysis showed that *INPP4B* (hazards ratio [HR]=1.457, 95% confidence interval [CI]: 1.003-2.115) affected the prognosis of CRC, and the Kaplan-Meier curve showed that patients with high *INPP4B* expression had shorter overall survival ($P<0.05$). χ^2 test was performed to analyze the relationship between INPP4B

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expression and clinicopathological indexes, and it was found that high expression of INPP4B was correlated with lymph node metastasis (χ^2=3.997, *P*=0.046) and neural invasion(χ^2=8.511, *P*=0.004). In *in vitro* experiments, CRC cells overexpressing *INPP4B* showed a significantly increased cell proliferation and migration compared to the cells in the control group (*P*<0.05). Analysis using the LinkedOmics database showed that *INPP4B* was correlated with extracellular matrix remodeling and cell migration. Pearson's correlation analysis showed that MMP7 was positively correlated with INPP4B (*r*=0.378 2, *P*<0.001). *INPP4B* overexpression or knockdown *in vitro* also led to the upregulation or the downregulation of *MMP7* expression in CRC cells. **Conclusion** INPP4B is highly expressed in CRC tissues and significantly correlated with lymph node metastasis, neural invasion, and patient prognosis. *MMP7* may mediate the role of INPP4B in promoting CRC cell migration and invasion.

【Key words】 INPP4B　Colorectal cancer　MMP7

结直肠癌(colorectal cancer, CRC)是全球肿瘤相关死亡的第二大原因[1]。在我国,2022年CRC新发病例59.2万,死亡病例30.9万,发病率居所有恶性肿瘤第2位,死亡率居第4位[2],CRC患者5年生存率为56.9%,远低于欧美国家[3],而转移性CRC的5年生存率不足20%[4]。因此,探明CRC的发生发展机制有助于改善临床治疗效果,改善CRC患者预后。

肌醇多磷酸4-磷酸酶Ⅱ型(inositol polyphosphate 4-phosphatase type Ⅱ B, INPP4B)是磷脂酰肌醇 3-激酶(phosphatidylinositol 3-kinase, PI3K)信号通路的关键激酶,募集并激活Akt的磷脂酰肌醇3, 4-二磷酸〔PI(3,4)P2〕去磷酸化,抑制Akt的活化,进而抑制Akt下游信号通路,抑制细胞的增殖、代谢、迁移等[5]。因此INPP4B通常被认为是通过负调节Akt活化来实现肿瘤抑制作用。但是有关INPP4B在CRC中的研究结果并不一致,有研究者表明INPP4B在肿瘤中表达下降,也有研究者报道INPP4B可以促进CRC的发生发展[6-7]。关于INPP4B对CRC细胞增殖的作用已有较多探讨,对CRC转移的作用尚缺乏研究。因此,需要进一步明确INPP4B在CRC中的作用以及探索INPP4B对CRC转移的影响。

基质金属蛋白酶7(matrix metallopeptidase 7, MMP7),又称为基质溶血素,可通过分解多种细胞外基质成分,促进癌症的转移和血管生成等[8]。MMP7被发现在CRC中呈高表达[9-10],尚没有研究表明INPP4B与MMP7的相关性及在CRC中的作用。

本研究拟通过生物信息学分析、临床样本的检测以及细胞水平的验证,明确INPP4B对CRC转移的影响,并明确CRC细胞中INPP4B与MMP7表达的相关性,为探究临床INPP4B高表达CRC患者发病机制提供新的思路。

1　资料与方法

1.1　公共数据集的分析

利用TIMER2.0数据库[11-13]分析*INPP4B*在肿瘤中的表达情况,采用Cox模型分析*INPP4B*、年龄、性别、种族、肿瘤纯度、肿瘤分期对结肠癌(colon adenocarcinoma, COAD)患者预后的影响,并做Kaplan-Meier曲线。用GEPIA2数据库[14](http://gepia2.cancer-pku.cn/#general)分析TCGA和GTEx数据库中癌和癌旁中*INPP4B*的表达情况。用LinkedOmics在线数据库[15](http://www.linkedomics.org/admin.php),选择TCGA-COAD/直肠癌(rectum adenocarcinoma, READ)数据队列,对组织分型为COAD的RNAseq数据集进行*INPP4B*的Pearson相关性分析。使用过表达富集分析法(ORA)富集*INPP4B*正相关基因的GO_BP。

1.2　临床CRC样本采集

收集2015年1月–2021年12月四川大学华西医院手术切除、临床及病理资料完整的102例样本,以及 26对癌(瘤)与癌旁(瘤旁)CRC样本。所有患者术前均未接受任何放、化疗及靶向治疗,所有标本均经两位病理医师复阅、确诊,并按WHO消化系统肿瘤分类标准确定病理分级及分期。本研究经四川大学华西医学中心医学伦理委员会批准(批准号KS2021548),样本收集已获得患者知情同意。

1.3　免疫组化分析

组织样本经固定、包埋、脱蜡、水化,过氧化氢溶液消除内源性过氧化物酶,后使用TRIS-EDTA(pH=9.0)对组织进行抗原修复。一抗INPP4B(CST, 14 543)稀释浓度为1：600,二抗为生物素化二抗,使用DAB显色,最后采用苏木素复染。显微镜下判读结果,每张切片随机选择5个高倍视野(×400)进行观察,每个视野随机计数100个细胞,按照着色程度评分:未着色为0分,浅黄色为1分,棕黄色为2分,深褐色为3分。单独评分两次,对两次评分求均值(IHC SCORE=1.215),将102例具有完整病理资料的样本按此均值分为INPP4B高表达组38例、INPP4B低表达组64例。

1.4　*INPP4B*过表达/敲减细胞系的构建

PCR扩增(TaKaRa Taq™)人*INPP4B*(*hINPP4B*)

(NM_001101669)的cDNA,采用双核酸限制性内切酶*Bam*HⅠ(NEB, R3136)和*Eco*RⅠ(NEB, R0101)、将cDNA插入过表达载体Lenti-EF1a-MCS-Flag.His-CMV-GFP-Puro(Addgene),构成Lenti-EF1a-*hINPP4B*-Flag.His-CMV-GFP-Puro质粒。使用BLOCK-iT™ RNAi Designer(https://rnaidesigner.thermofisher.com/rnaiexpress/)设计shRNA序列并合成(擎科生物),退火后形成双链DNA。采用双核酸限制性内切酶*Bam*HⅠ和*Eco*RⅠ、敲减载体Lenti-U6-shNT-SV40-GFP-Puro(Addgene),插入合成的双链shRNA,连接后构建Lenti-U6-sh*INPP4B*-SV40-GFP-Puro。用DH5α大肠杆菌(tolobio)转化后摇菌、提质粒。将构建好的质粒及其阴性对照与慢病毒包装辅助质粒psPAX2和Pmd2.G与转染试剂PEI(MCE)混合后转染HEK293T细胞(Harvard Medical School)。收集24 h 、48 h的转染细胞上清液,过滤后加入Polybrene(10 μg/mL)(MCE),将病毒转染进HCT8、HKe3细胞(山西省肿瘤医院),转染完成后用Puromycin(6 μg/mL)(APExBIO)进行筛选。获得*INPP4B*过表达细胞株$HCT8^{INPP4B\text{-}OE}$和*INPP4B*敲减细胞株$HKe3^{INPP4B\text{-}KO}$及对应的空质粒对照细胞株$HCT8^{CTL\text{-}OE}$、$HKe3^{CTL\text{-}KO}$。

1.5 Western blot

用RIPA蛋白裂解液(Beyotime)裂解细胞,提取总蛋白,SDS-PAGE凝胶电泳。蛋白定量采用Bradford法。检测抗体为INPP4B(CST, 4039),β-actin(Sigma-Aldrich, ABT1485)用作内参,二抗Anti rabbit IgG(CST, 7074),Anti Mouse IgG(CST, 7076),ECL 曝光试剂盒(Cytiva, RPN2235),ImageQuant LAS 4000化学发光成像分析仪(GE healthcare)。

1.6 实时荧光定量PCR

用TRIZOL(Ambion)裂解细胞沉淀,加入氯仿后离心,收集上层水相到无RNase管中,加入异丙醇离心沉淀RNA。乙醇清洗,无酶水溶解后nanodrop(Thermo)测量RNA浓度。使用逆转录试剂盒(Promega, A2971)将RNA逆转录为cDNA,qPCR试剂盒(Promega)检测目的基因Ct值。引物由擎科生物公司合成,序列见表1,反应程序如下:95 ℃ 2 min , 1个循环;95 ℃ 15 s, 60 ℃ 30 s, 40个循环。以*GAPDH*作为内参,使用$2^{-\Delta\Delta Ct}$计算基因相对表达量,实验重复3次。

1.7 细胞划痕实验

将对数生长期的HCT8、HKe3细胞胰酶消化后离心重悬,计数后接种于6孔板中。敷箱过夜,第二日垂直地在板孔中央划线,PBS清洗3次,洗去划落的细胞,加入无血清培养基2 mL,放入培养箱。分别于0、12、24 h选取固定视野进行拍照并进行分析。

表1 引物序列

Table 1 Primer sequences

Gene name	Primer sequences (5' to 3')	Product length/bp
INPP4B	F: CTGATGCTGACGCTAAGAAGAG	108
	R: TAGGAAGCCTGGGTCATACA	
MMP7	F: GCTGACATCATGATTGGCTTTGCG	238
	R: CTGCATTAGGATCAGAGGAATGTCCC	
GAPDH	F: GGTGTGAACCATGAGAAGTATGA	123
	R: GAGTCCTTCCACGATACCAAAG	

INPP4B: inositol polyphosphate 4-phosphatase type Ⅱ B; *MMP7*: matrix metallopeptidase 7; *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase.

1.8 实时无标记动态细胞分析技术(RTCA)

将165 μL含有10%FBS的DMEM细胞培养基加入到CIM-plate的下室中,在预先铺设好基质胶的CIM-plate上室孔中加入100 μL混合均匀的无血清各分组细胞悬液,细胞密度为4×10^4 mL^{-1},加好后的CIM板置于超净台中室温放置30 min后放到培养箱中的RTCA仪器(Agilent, xCELLigence RTCA DP)上以进行连续阻抗记录。通过每15 min连续阻抗记录测量24 h的CELL INDEX值反映细胞侵袭迁移能力。CELL INDEX值越高,细胞侵袭、迁移能力越高。

1.9 细胞增殖实验

胰酶消化对数生长期的细胞,计数后重悬,以4×10^3个/孔接种于96孔板,分别在接种细胞后的1、3、5、7 d每孔加入15 μL CellTiter 96® AQueous One试剂(Promega)和100 μL培养基,敷箱孵育2 h ,酶标仪检测490 nm处的吸光度值。吸光度值正比于细胞增殖能力。

1.10 统计学方法

采用GraphPad Prism 8软件(GraphPad Software, San Diego, CA, USA),多组间比较采用方差分析(ANOVA),组间两两比较使用*t*检验。采用IBM SPSS Statistics V21.0中χ^2检验分析INPP4B高低表达两组间临床病理指标。使用TIMER2.0数据库的患者资料,以Cox比例风险模型探究CRC的危险因素,利用Kaplan-Meier法分析*INPP4B*与CRC患者终点事件的关系。使用LinkedOmics中的Pearson相关性分析探究*INPP4B*与*MMP7*的相关性。$P<0.05$为差异有统计学意义。

2 结果

2.1 公共数据集初步筛选

2.1.1 *INPP4B*在CRC肿瘤组织高表达

使用GEPIA2数据库结合TCGA和GTEx的数据分析*INPP4B*表达情况,结果显示*INPP4B*在CRC中的表达高于正常样本(图1A)。

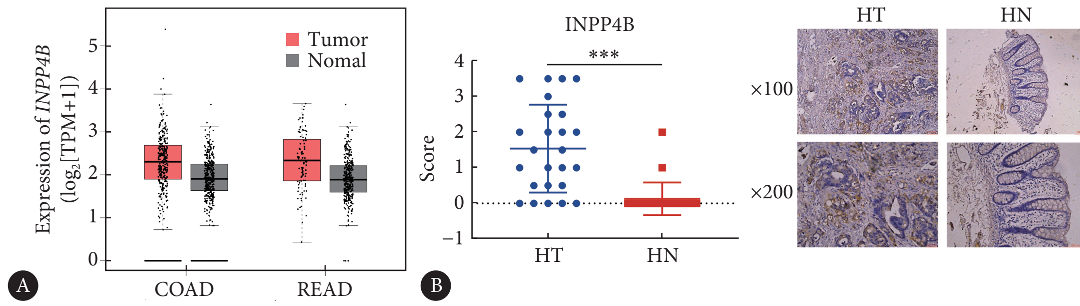

图 1 INPP4B在CRC组织中的表达情况

Fig 1 Expression of INPP4B in CRC cancer tissue samples

A, The expression of *INPP4B* in COAD (275 in tumor group and 349 in nomal controls) and READ (92 in tumor group and 318 in nomal controls) was analyzed using the GEPIA2 database combined with TCGA and GTEx data; B, the expression of INPP4B was determined by IHC in 26 pairs of colorectal tumor tissue and matching normal tissue samples (*** P<0.001). COAD: colon adenocarcinoma; READ: rectal adenocarcinoma; TPM: transcripts per kilobase million; HT: human tumor; HN: human normal.

本研究在临床收集到26例成对结直肠肿瘤组织与相应正常组织样本，IHC检测INPP4B表达情况。结果发现INPP4B在结直肠肿瘤中的表达情况明显高于肿瘤旁正常组织(图1B)。

2.1.2　*INPP4B*与CRC的生存预后相关

Cox模型显示(表2)，在TIMER2.0数据库完成随访的258例COAD患者中(包括64例死亡)，*INPP4B*的表达[风险比(hazard ratio, HR)=1.457，95%可信区间(confidence interval, CI)：1.003～2.115]，年龄(HR=1.027，95%CI：1.004～1.050)以及分期(stage 4)(HR=7.030，95%CI：2.366～20.894)与患者生存预后有关(P<0.05)。

表 2　Cox模型评估TIMER2.0数据库中*INPP4B*对COAD生存的影响(*n*=258)

Table 2　Using the Cox model to evaluate the effect of *INPP4B* on the overall survival of COAD (*n*=258)

Variable	HR	95% CI	P
INPP4B expression			
No	1	Ref	
Yes	1.457	1.003-2.115	0.048
Purity	0.879	0.271-2.855	0.830
Age	1.027	1.004-1.050	0.023
Sex			
Female	1	Ref	
Male	1.279	0.755-2.167	0.360
Race			
Others	1	Ref	
Black	0.611	0.124-3.019	0.545
White	0.578	0.128-2.611	0.476
Stage			
Stage 1	1	Ref	
Stage 2	1.317	0.437-3.966	0.625
Stage 3	2.192	0.746-6.440	0.154
Stage 4	7.030	2.366-20.894	<0.001

HR: hazard ratio; CI: confidence interval.

根据肠上皮*INPP4B*表达水平的中位数将患者分为两组，比较高表达组和低表达组病例的生存期，结果提示高表达*INPP4B*的COAD患者生存较差(图2)。

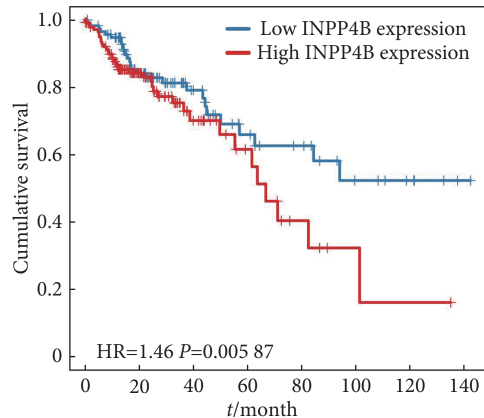

图 2 TIMER2.0数据库中不同*INPP4B*表达水平COAD患者的生存预后

Fig 2 Prognosis for the survival of COAD patients with different expression levels of *INPP4B* in the intestinal epithelium

n=129 per group.

2.2　临床验证INPP4B与临床病理指标的关系

根据INPP4B免疫组化评分均值，将102个临床样本分为INPP4B HIGH组(n=38)和LOW组(n=64)，比较两组间各项临床病理指标的差异。结果发现INPP4B与肿瘤淋巴结转移(χ^2=3.997，P=0.046)、神经浸润(χ^2=8.511，P=0.004)有关，INPP4B高表达的样本肿瘤淋巴结转移和神经浸润都明显增多(表3)。分析结果并未发现INPP4B与性别、年龄、侵袭程度、分化程度以及肿瘤发生部位有关(P>0.05)。

2.3　体外细胞验证INPP4B高表达对CRC细胞增殖、迁移和侵袭的促进

2.3.1　过表达和敲低细胞株的验证

与空质粒转染细胞HCT8$^{CTL\text{-}OE}$相比，过表达*INPP4B*的

表 3 102例临床CRC组织中INPP4B表达与临床病理指标的关系

Table 3 The relationship between INPP4B expression and clinical pathological indicators in 102 clinical tissue samples of CRC

Pathological feature	Total	INPP4B expression/case (%)		χ^2	P
		High	Low		
Sex				1.213	0.268
Female	60	25 (41.67)	35 (58.33)		
Male	42	13 (30.95)	29 (69.05)		
Age				1.063	0.301
≤60 yr.	47	15 (31.91)	32 (68.09)		
>60 yr.	55	23 (41.82)	32 (58.18)		
Tumor differentiation				2.982	0.225
Low	27	13 (48.15)	14 (51.85)		
Middle	49	19 (38.78)	30 (61.22)		
High	21	5 (23.81)	16 (76.19)		
Tumor invasion				0.601	0.435
T1-T2	28	9 (32.14)	19 (67.86)		
T3-T4	69	28 (40.58)	41 (59.42)		
Lymph node metastasis				3.997	0.046
+	39	19 (48.72)	20 (51.28)		
−	62	18 (29.03)	44 (70.97)		
Neural invasion				8.511	0.004
+	18	12 (66.67)	6 (33.33)		
−	83	25 (30.12)	58 (69.88)		
Anatomic subdivision of the neoplasm				0.210	0.647
Colon	54	19 (35.19)	35 (64.81)		
Rectum	48	19 (39.58)	29 (60.62)		

细胞HCT8$^{INPP4B\text{-}OE}$的INPP4B在基因和蛋白水平表达均升高(图3A)。敲低*INPP4B*表达的HKe3$^{INPP4B\text{-}KO}$细胞与对照细胞HKe3$^{CTL\text{-}KO}$相比，INPP4B表达水平降低(图3A)。

2.3.2 细胞增殖

通过CellTiter-Glo®发光法检测细胞增殖活力，观察不同INPP4B表达水平对CRC细胞增殖的影响。结果显示，过表达*INPP4B*细胞HCT8$^{INPP4B\text{-}OE}$增殖相比于对照组HCT8$^{CTL\text{-}OE}$明显增高，而敲低*INPP4B*表达细胞HKe3$^{INPP4B\text{-}KO}$增殖能力显著受到抑制(图3B)。

2.3.3 细胞迁移和侵袭

划痕实验结果显示(图4A)，*INPP4B*过表达细胞HCT8$^{INPP4B\text{-}OE}$相比于对照组HCT8$^{CTL\text{-}OE}$迁移距离增加，与之一致的是敲低*INPP4B*表达细胞HKe3$^{INPP4B\text{-}KO}$相比于对照组HKe3$^{CTL\text{-}KO}$迁移距离下降。

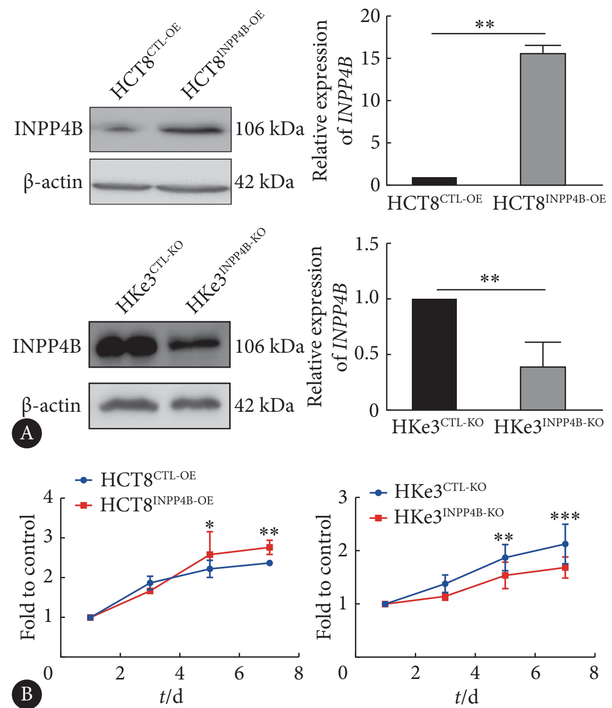

图 3 *INPP4B*过表达促进CRC细胞增殖

Fig 3 *INPP4B* overexpression promotes the proliferation of CRC cells

A, Western blot (left) and RT-PCR (n=3 per group, right) were performed to verify INPP4B expression in transfected cells; B, Cell Titer-Glo® was used to detect the effect of different levels of INPP4B expression on cell proliferation (n=8 per group). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

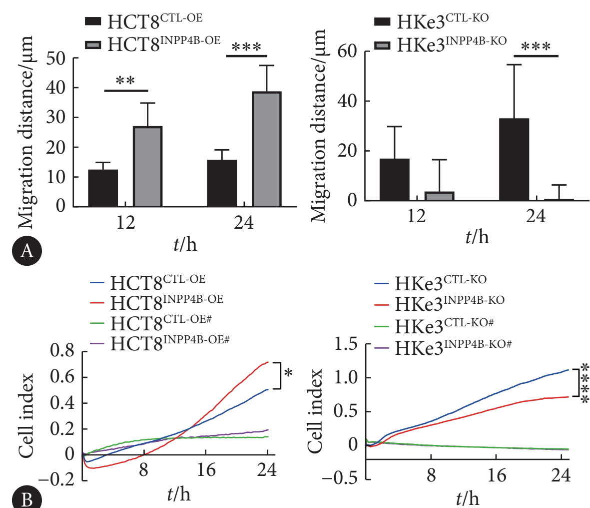

图 4 *INPP4B*过表达促进CRC细胞迁移和侵袭

Fig 4 The overexpression of *INPP4B* promotes the migration and invasion of CRC cells

A, Wound healing assay was conducted to measure the migration of *INPP4B* overexpression (n=8 per group) and knockdown (n=10 per group) cell lines; B, RTCA assay was conducted to determine the invasion of *INPP4B* overexpressing and knockdown cell lines (n=3 per group). # Control (serum-free). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$.

RTCA检测结果显示(图4B)，HCT8$^{INPP4B\text{-}OE}$细胞相比

于对照组HCT8^CTL-OE^的侵袭迁移能力增强，HKe3^INPP4B-KO^细胞相比于对照组HKe3^CTL-KO^的侵袭迁移能力下降。

2.4 *INPP4B*促进CRC细胞迁移与细胞外基质重塑有关

为进一步研究*INPP4B*促进细胞迁移的机制，本研究利用LinkedOmics在线数据进行基因功能分析，结果显示与*INPP4B*正相关的基因富集的前3个通路分别是细胞外基质组成和结构、细胞迁移的正向调控，提示*INPP4B*调控CRC转移与重塑细胞外基质高度相关(附图1)。Pearson相关分析的基因热图显示了前50个与*INPP4B*正相关基因(附图2)，在前10个正相关基因中，*MMP7*与*INPP4B*表达水平正相关(r=0.378 2, P<0.01)(图5A)。所有附图请见网络资源附件。

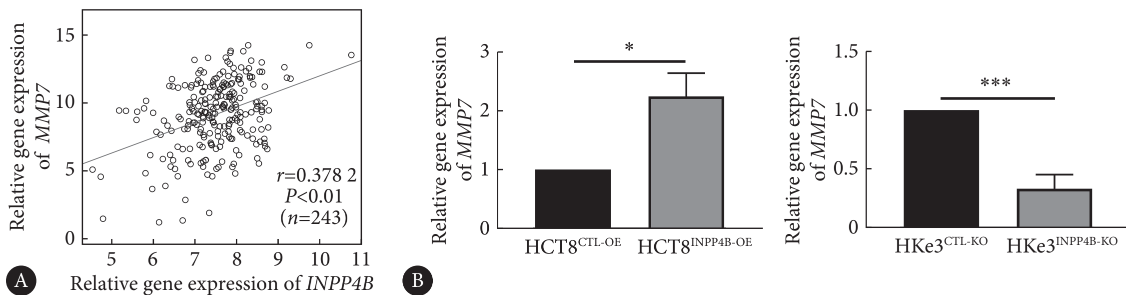

图 5 CRC的*INPP4B*表达与*MMP7*正相关

Fig 5 The expression of *INPP4B* is positively correlated with *MMP7* in CRC

A, Pearson's correlation analysis was performed to analyze the correlation between *INPP4B* and *MMP7*; B, qPCR was performed to measure the expression of *MMP7* after overexpression (n=3 per group) or knockdown (n=4 per group) of *INPP4B* in CRC cells. $^*P$<0.05, $^{***}P$<0.001.

本研究进一步通过qPCR检测*INPP4B*过表达或敲减细胞中*MMP7*的表达，结果如图5B所示。与对照相比，过表达*INPP4B*促进了*MMP7*的表达，而*INPP4B*表达水平的下调抑制了*MMP7*的表达。

3 讨论

在结肠癌发生发展过程中，PI3K通路的激活具有重要意义[16]。CRC患者常见的遗传和表观遗传异常，例如EGFR的耐药，*KRAS*基因突变，*BRAF*以及*PTEN*的缺失，都与PI3K信号的激活高度相关[17-18]。PI3K信号的激活受到肌醇多磷酸酶的负调节。其中INPP4B这种脂质磷酸酶催化PI(3,4)P2的4位点去磷酸化降解为PI(3)P，从而终止PI3K信号。

虽然具有类似功能的PI3K信号的负性调节分子*PTEN*是一个被公认的抑癌基因，但有关 INPP4B在不同肿瘤中功能的研究结果却截然相反。在三阴性乳腺癌和基底样乳腺癌中INPP4B杂合性缺失的现象频发，通过延缓EGFR的降解使得Akt信号通路的作用时间延长和直接促使Akt磷酸化增加来促进癌症的发生[19-20]。INPP4B的缺失可使良性甲状腺瘤转变为致死性和转移性滤泡样甲状腺癌[21]。INPP4B蛋白在前列腺癌组织中表达下调[22]，并且通过抑制PKC信号和Akt活性抑制前列腺癌的侵袭[23-24]。但INPP4B在不同组织来源的肿瘤中并不都表现为一个抑癌基因。在急性髓系白血病(acute myelogenous leukemia, AML)中促进细胞的增殖[25]同时还介导了AML的耐药机制[26]。

尽管INPP4B对肿瘤增殖和转移的影响已在多种癌症中进行了研究，但INPP4B在CRC中的功能尚未明确，目前的研究多聚焦于对CRC细胞增殖的研究。GUO等[6]在细胞水平上研究发现INPP4B活化结肠癌细胞中PI3K和SGK3信号，提示*INPP4B*作为癌基因促进肠上皮的异常增殖[27]。但也有研究报道INPP4B在原发性和转移性CRC组织中的表达相比与正常肠黏膜的表达降低[7]。在一项CRC干细胞研究中，INPP4B促进了高度转移性CRC干细胞样细胞(CR-CSLCs)的致瘤性[28]，提示了INPP4B和转移的关系，却缺乏进一步的实验验证。

为明确INPP4B在CRC中的作用，本研究利用多个在线数据库进行生物信息学分析*INPP4B*在肿瘤和正常组织中的表达与CRC患者预后等关系，从基因表达层面系统挖掘*INPP4B*与CRC中进展相关的候选基因集，并基于临床组织和体外细胞培养水平进行验证。无论从生物信息学、临床样本还是细胞水平实验都发现INPP4B促进了CRC的转移。通过TCGA RNA-seq数据集探索CRC与*INPP4B*表达水平相关基因，以及GO富集*INPP4B*正相关的基因所在通路，本研究发现*INPP4B*与细胞外基质组成和结构的高度相关，在这些通路中*MMP7*分子被富集，同时又与*INPP4B*的表达具有明显的正相关。

MMP7是基质金属蛋白酶家族的一员，参与前列腺癌[29]、乳腺癌[30]等肿瘤的转移。在CRC中发挥着重要的作用，与细胞增殖、迁移和侵袭，以及血管形成相关[8]。

研究表明MMP7因促进CRC的转移[31],是CRC上皮间质转化的标志物[32],被视为CRC的潜在具有预后价值的标志分子[33]。MMP7还与神经浸润相关[34],这与本研究结果一致。除了参与肿瘤的转移,MMP7在CRC的结肠腺瘤患者血清和组织中升高[35],可导致CRC的发生,可能是腺瘤-癌序列中的主要事件[35-36]。MMP7还可激活其他的MMPs,例如MMP1、MMP2、MMP8、MMP9[37-38],进而参与肿瘤转移、生长等过程。因此,MMP7在CRC发生发展中有重要作用。

然而,MMP7在CRC中的调控机制目前仍不明确。研究表明,PI3K/Akt通路与MMP7表达密切相关。CRC中,PI3K/Akt通路的抑制剂以及Akt的沉默都影响了癌细胞中MMP7的表达,从而影响肿瘤的转移[39-40]。两者之间的关系也在胃癌[41]、非小细胞肺癌[42]和卡希波肉瘤[43]中得到验证。INPP4B作为PI3K/Akt通路的关键调节因子,很有可能参与了MMP7的调节。本研究在体外细胞水平也证实INPP4B可正向调控MMP7的表达,需要进一步研究明确INPP4B调控MMP7的机制。

综上,本研究明确了INPP4B在CRC中高表达与肿瘤转移、患者预后相关。INPP4B高表达重塑细胞外基质,促进了CRC细胞的转移。

* * *

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Author Contribution LAI Meng is responsible for conceptualization, data curation, formal analysis, writing--original draft, and writing--review and editing. MAO Zhigang is responsible for resources and supervision. TANG Deng and LAN Siqi are responsible for investigation and methodology. YAN Ruiting, XIANG Qi, and ZHAO Xianxian are responsible for investigation and validation. SU Mi and WANG Yufang are responsible for conceptualization, data curation, funding acquisition, writing--original draft, 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 take responsibility for all aspects of the work.

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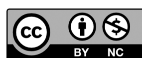

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