四川大学学报（医学版） 2024，55（3）：731－738
J Sichuan Univ（Med Sci） doi: 10.12182/20240560403

在线全文

miR-342-3p通过靶向抑制PPM1E促进肾透明细胞癌细胞的增殖、迁移和侵袭*

王荦楠， 李卓然， 吴洁清， 谢晋玲
新乡医学院第一附属医院 肿瘤内科一病区（新乡 453100）

【摘要】 目的 探究微小RNA-342-3p/$Mg^{2+}Mn^{2+}$依赖的蛋白磷酸酶1E（miR-342-3p/PPM1E）轴对肾透明细胞癌（clear cell renal cell carcinoma, ccRCC）细胞增殖、迁移和侵袭的影响。 **方法** 搜索基因芯片GSE12105、GSE23085、GSE66271及GSE66270，分析miR-342-3p、PPM1E与ccRCC临床恶性表型的关系。miR-342-3p inhibitor转染ACHN、769-P细胞，检测miR-342-3p对细胞增殖、迁移和侵袭的影响；构建稳定高表达miR-342-3p的ACHN细胞系，并观察其在Balb/c裸鼠体内的成瘤情况；双萤光素酶报告基因验证miR-342-3p与*PPM1E*的靶向关系，同时转染miR-342-3p mimic、pcDNA-*PPM1E*质粒，观察PPM1E是否可逆转miR-342-3p过表达对细胞增殖、迁移和侵袭的影响。 **结果** miR-342-3p在ccRCC中表达上调，且在不同T分期、G分期、预后患者中存在差异表达（$P<0.05$）；miR-342-3p高表达组总生存期明显低于miR-342-3p低表达组（$P<0.05$）。与miR-NC组相比较，inhibitor组miR-342-3p水平显著下调，细胞增殖能力、迁移细胞数和侵袭细胞数显著降低（$P<0.05$）；与miR-NC组相比较，miR-342-3p组肿瘤组织体积及质量、miR-342-3p水平均明显升高，*PPM1E* mRNA水平明显降低（$P<0.05$）。PPM1E在ccRCC中表达下调，在不同M分期、N分期、G分期及复发情况患者中存在差异表达（$P<0.05$）。miR-342-3p可靶向抑制PPM1E表达，与miR-NC组相比较，miR-342-3p组细胞增殖能力、迁移细胞数和侵袭细胞数明显升高（$P<0.05$），而PPM1E可逆转miR-342-3p mimic对ccRCC细胞的促进作用（$P<0.05$）。 **结论** miR-342-3p可靶向抑制PPM1E表达，从而促进ccRCC细胞增殖、迁移和侵袭。

【关键词】 肾透明细胞癌 miR-342-3p $Mg^{2+}Mn^{2+}$依赖的蛋白磷酸酶1E 增殖 迁移 侵袭

miR-342-3p Promotes the Proliferation, Migration, and Invasion of Clear Cell Renal Cell Carcinoma Cells by Targeted Inhibition of PPM1E

WANG Luonan, LI Zhuoran, WU Jieqing, XIE Jinling. The First Ward, Department of Oncology, the First Affiliated Hospital of Xinxiang Medical University, Xinxiang 453100, China

【Abstract】 Objective To explore the effects of microRNA-342-3p/$Mg^{2+}Mn^{2+}$-dependent protein phosphatase 1E (miR-342-3p/PPM1E) on the proliferation, migration, and invasion of clear cell renal cell carcinoma (ccRCC) cells. **Methods** The gene chips GSE12105, GSE23085, GSE66271, and GSE66270 were searched, and the relationship between miR-342-3p, PPM1E, and the clinical malignant phenotypes of ccRCC was analyzed. ACHN and 769-P cells were transfected with miR-342-3p inhibitor. The effects of miR-342-3p on cell proliferation, migration, and invasion were examined. ACHN cell line with stable and high expression of miR-342-3p was constructed, and the tumorigenicity of the cell line in BALB/c nude mice was observed. The targeted relationship between miR-342-3p and *PPM1E* was verified by dual-luciferase reporter gene assay. The cells were transfected with miR-342-3p mimic and pcDNA-*PPM1E* plasmids to observe whether PPM1E could reverse the effects of miR-342-3p overexpression on the proliferation, migration, and invasion of the cells. **Results** The expression of miR-342-3p was upregulated in ccRCC, and there were significant differences among patients with tumors of different T stages and G stages and those with different prognoses ($P<0.05$). The overall survival in the miR-342-3p high-expression group was significantly shorter than that in the low-expression group ($P<0.05$). Compared with those in the miR-NC group, the miR-342-3p level was significantly downregulated in the inhibitor group, and the cell proliferation ability and the numbers of migrating and invading cells were also significantly decreased ($P<0.05$). Compared with the miR-NC group, miR-342-3p group had significantly increased volume and mass of tumor tissues and miR-342-3p level, but significantly decreased level of *PPM1E* mRNA ($P<0.05$). The expression of PPM1E was downregulated in ccRCC, and there were significant differences among patients with tumors of different M stages, N stages, and G stages, and different recurrence statuses ($P<0.05$). The miR-342-3p could inhibit the expression of PPM1E in a targeted way. Compared with the miR-NC group, the miR-342-3p group had significantly increased cell proliferation ability and increased numbers of migrating and invading cells ($P<0.05$). However, PPM1E could reverse the promotion effect of miR-342-3p mimic on ccRCC cells ($P<0.05$). **Conclusion** The miR-342-3p can inhibit PPM1E expression in a targeted way, and thus promotes the proliferation, migration, and invasion of ccRCC cells.

【Key words】 Clear cell renal cell carcinoma miR-342-3p $Mg^{2+}Mn^{2+}$-dependent protein phosphatase 1E Proliferation Migration Invasion

* 2022年度河南省医学科技攻关计划联合共建项目（No. LHGJ2022062）资助
出版日期：2024-05-20

肾癌是常见的泌尿系恶性肿瘤,其中70%~75%的病理类型均为肾透明细胞癌(clear cell renal cell carcinoma, ccRCC)[1]。目前,对于局限性ccRCC术后尚无标准辅助治疗方案,而对于转移性ccRCC尚无标准治疗方案[2]。因此,加强ccRCC的基础研究,寻求新型治疗手段尤为重要。微小RNA(microRNAs, miRNAs)在恶性肿瘤中发挥重要的调控作用,且根据靶基因的表达作为促癌因子或抑癌因子[3]。据报道,miR-342-3p在卵巢癌、肝癌等恶性肿瘤中发挥促癌/抑癌作用[4-5]。但miR-342-3p在ccRCC中的作用和机制尚不明确。蛋白磷酸酶可通过催化底物蛋白的去磷酸化反应,参与生物体内多种信号转导途径。$Mg^{2+}Mn^{2+}$依赖的蛋白磷酸酶1E(protein phosphatase 1E, PPM1E)为蛋白磷酸酶成员之一。研究显示,PPM1E的表达与胃癌细胞增殖、乳腺癌临床恶性表型相关[6-7]。本研究通过starBase预测*PPM1E* mRNA的3'-UTR与miR-342-3p存在潜在结合位点,探究miR-342-3p是否通过直接靶向*PPM1E* 3'-UTR影响ccRCC细胞增殖、迁移与侵袭过程,以期为临床ccRCC的诊治提供指导。

1 材料与方法

1.1 细胞与实验动物

人ccRCC细胞ACHN细胞、769-P细胞(上海通派生物科技有限公司);10只5~6周龄雄性Balb/c裸鼠,体质量16~20 g,动物生产许可证号:SCXK(京)2019-0041;本研究由新乡医学院第一附属医院动物伦理委员会批准,批准号(2022)伦药会审字(50)号。

1.2 试剂

miR-342-3p mimic、miR-342-3p inhibitor、miR-NC、pcDNA *PPM1E*质粒(上海联迈生物工程有限公司,中国);miR-342-3p引物、*PPM1E*引物(上海生工生物工程股份有限公司,中国);脂质体2000试剂盒、RPMI-1640培养基、胎牛血清(Thermo Fisher Scientific,美国);细胞计数试剂盒(CCK-8)、双萤光素酶报告基因检测试剂盒(上海碧云天生物技术有限公司,中国);基质胶、转移小室(BD Biosciences,美国);PrimeScript RT reagent kit(大连宝生物工程有限公司,中国);PPM1E抗体、β-actin抗体(Abcam,美国)。

1.3 方法

1.3.1 数据提取及分析

于GEO网站搜索基因芯片GSE12105、GSE23085、GSE66271及GSE66270,用GEO 2R识别ccRCC组织和正常肾组织之间的差异表达基因,下载TCGA_ccRCC数据和临床信息,分析miR-342-3p、PPM1E与ccRCC临床恶性表型的关系。分析TCGA_ccRCC矩阵中,与无远处转移(M0)组患者相比,有远处转移(M1)组患者中表达下调的基因。

1.3.2 细胞培养与转染

将ACHN、769-P细胞用含有10%胎牛血清的RPMI-1640培养基培养,置于37 ℃、体积分数5% CO_2温箱,利用0.25%胰酶进行传代培养,采用脂质体2000试剂盒,分别以miR-342-3p inhibitor及其阴性对照物(miR-NC)、pcDNA-*PPM1E*质粒转染细胞,转染48 h后收集细胞,检测miR-342-3p、*PPM1E*表达。转染miR-342-3p质粒及其对照miR-NC,用G418筛选出稳定过表达miR-342-3p的ACHN和769-P细胞系。

1.3.3 实时荧光定量PCR检测miR-342-3p、*PPM1E* mRNA水平

收集各组细胞,采用Trizol试剂提取总RNA,逆转录合成cDNA,再通过PrimeScript RT reagent kit进行mRNA水平检测,分别以*U6*和*GAPDH*为内参,采用$2^{-\Delta\Delta Ct}$法计算miR-342-3p、*PPM1E* mRNA水平。miR-342-3p上游引物为:5'-GGGTCTCACACAGAAATCGC-3',下游引物为:5'-CAGTGCGTCTCGTGGAGT-3';*PPM1E*上游引物为:5'-GGAGTAGATGCTGCTATTTATG-3',下游引物为:5'-TAGCTCTGGAAACCGCACA-3';*U6*上游引物为:5'-CTCGCTTCGGCAGCACA-3',下游引物为:5'-AACGCTTTGGTATCGTGGAAGG-3';*GAPDH*上游引物为:5'-ACAACTTTGGTATCTGTGGAGG-3',下游引物为:5'-GCGATCACGCCACAGTTTC-3'。

1.3.4 细胞增殖实验

将各组细胞以5×10^3细胞/孔接种于96孔板,分别培养24、48、72、96、120 h,加入CCK-8试剂后,继续孵育4 h,酶标仪于450 nm波长处检测各孔吸光密度值(optical density, OD),以OD值表示细胞增殖能力。

1.3.5 Transwell小室实验

将Transwell小室放入24孔板,侵袭能力检测需要预先用基质胶包被转移小室上室,迁移能力检测不需要包被基质胶;将各组细胞以1×10^5/mL接种于小室上室,下室添加含有10%胎牛血清的培养基,培养48 h,取出小室用体积分数95%乙醇固定细胞,并用质量浓度0.1%结晶紫染色,光镜下拍照并计数。

1.3.6 移植瘤模型的建立

取稳定过表达miR-342-3p及其对照组ACHN细胞,调整细胞密度为1×10^7/mL,取0.2 mL细胞悬液接种于Balb/c裸鼠背部皮下,每组5只,观察肿瘤生长情况,待瘤体形成时,测量其长径、短径,计算肿瘤体积,绘制肿瘤生长曲线。观察结束时(14 d)安乐死裸鼠,剖取肿瘤组织,称

质量、测量体积, 实时荧光定量PCR检测miR-342-3p水平。

1.3.7　双萤光素酶报告基因检测

通过starBase预测miR-342-3p在*PPM1E* mRNA的3'-UTR处存在结合位点, 合成含有与miR-342-3p结合的野生型(WT)或突变型(MT)的*PPM1E* 3'-UTR序列, 并克隆至pGL-3萤光素酶报告基因载体上, 将萤光素酶报告基因载体与miR-342-3p mimic、miR-NC共转染ACHN细胞, 48 h后收集各组细胞, 通过双萤光素酶报告基因检测试剂盒检测萤光素酶活性。

1.3.8　蛋白印迹检测PPM1E蛋白表达

收集各组细胞, 采用RIPA试剂分离总蛋白, 检测蛋白浓度, 各组取50 μg蛋白经10% SDS-PAGE凝胶电泳, 然后将蛋白转移至PVDF膜, 浸于5%脱脂牛奶中封闭2 h后, 分别添加1∶1 000稀释的一抗PPM1E、β-actin, 置于4 ℃振荡孵育过夜, 次日加入1∶5 000稀释的HRP标记的二抗, 37 ℃振荡孵育1 h, 最后添加ECL发光试剂检测蛋白质表达, 结果表示为PPM1E与β-actin灰度值比值。

1.4　统计学方法

采用软件SPSS17.0进行统计学分析, 定量数据以 $\bar{x} \pm s$ 表示, 所有实验均重复3次。两组均数间比较采用*t*检验, 多组均数比较采用单因素方差分析, 进一步两两比较采用LSD检验, $P<0.05$为差异有统计学意义。

2　结果

2.1　miR-342-3p与ccRCC临床恶性表型的关系

见图1。下载GSE12105和GSE23085数据矩阵, 维恩图展示数据矩阵中差异miRNA个数和关系, GSE12105有46个差异miRNA, GSE23085有81个差异miRNA, 其中

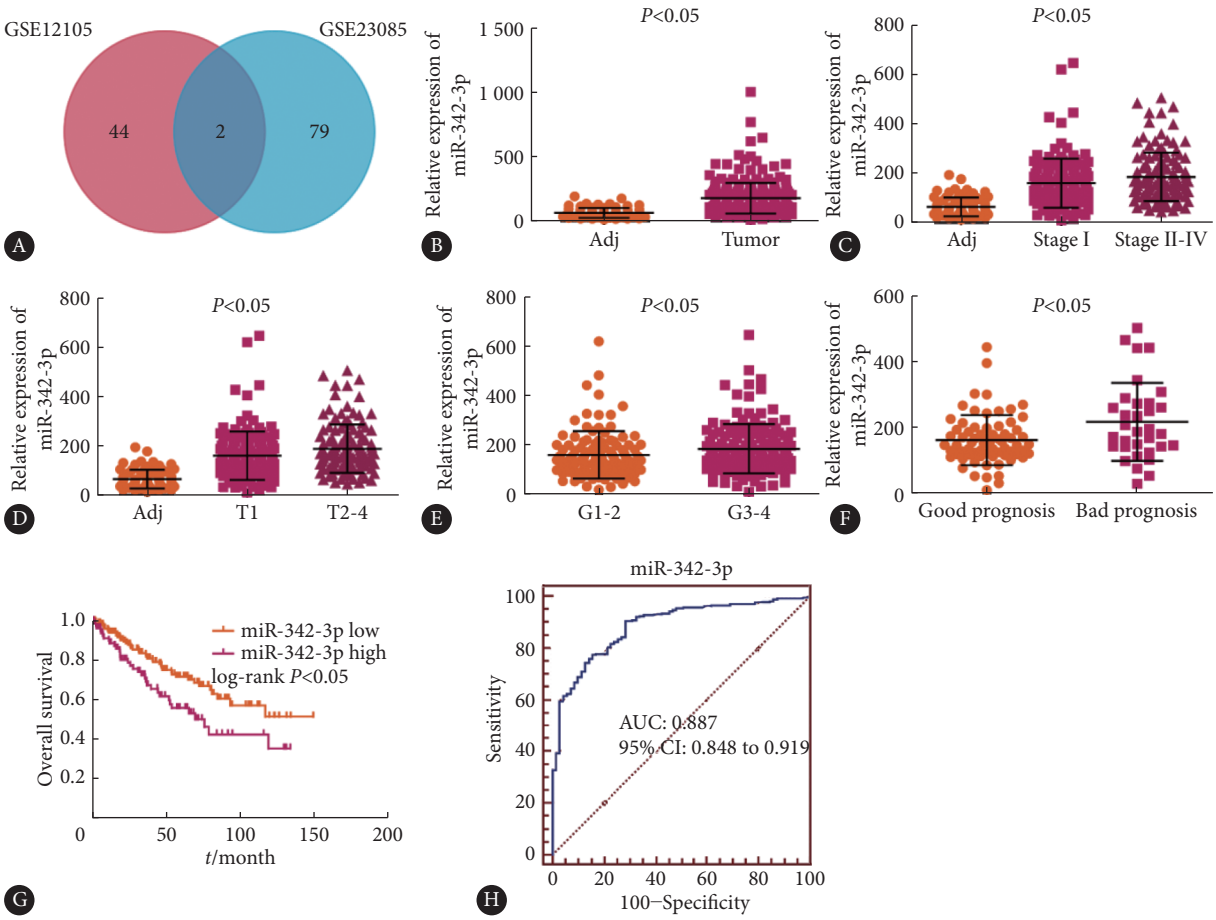

图 1　miR-342-3p与ccRCC临床恶性表型的关系

Fig 1　Relationship between miR-342-3p and the clinical malignant phenotypes of ccRCC

A, miRNAs analysis of the differences between GSE12105 and GSE23085 data matrices; B, comparison of the miR-342-3p levels in ccRCC tissues (Tumor group, *n*=261) and normal para-carcinoma tissues (Adj group, *n*=71); C, comparison of the miR-342-3p levels in patients with tumors of Adj (*n*=71), stage I (*n*=132) and stages Ⅱ-Ⅳ (*n*=121); D, comparison of the miR-342-3p levels in patients with tumors of Adj (*n*=71), stage T1 (*n*=136) and stages T2-4 (*n*=119); E, comparison of the miR-342-3p levels in patients with tumors of grades G1-2 (*n*=109), grades G3-4 (*n*=143); F, comparison of the miR-342-3p levels in patients with good prognosis (*n*=69) and bad prognosis (*n*=33); G, comparison of the overall survival in patients with low level (*n*=170) and high level (*n*=85) of miR-342-3p; H, diagnostic efficiency of miR-342-3p for ccRCC.

miR-342-3p和miR-15a-5p表达上调(图1A)。下载TCGA_ccRCC数据和临床信息,分析miR-342-3p和miR-15a-5p与ccRCC恶性临床表型关系。结果发现miR-15a-5p与ccRCCG分期无明显相关性,而miR-342-3p在ccRCC组织和癌旁正常组织、不同T分期、G分级、预后患者中存在差异表达($P<0.05$,图1B ~ 1F)。miR-342-3p表达水平影响ccRCC患者的生存状态,miR-342-3p高表达组总生存期明显低于miR-342-3p低表达组($P<0.05$,图1G)。ROC曲线分析显示,miR-342-3p诊断ccRCC的曲线下面积为0.887(95%置信区间为0.848 ~ 0.919,图1H)。

2.2 miR-342-3p低表达对ccRCC细胞增殖、迁移和侵袭的影响

转染ACHN、769-P细胞后,与miR-NC组比较,inhibitor组miR-342-3p水平显著下调,细胞增殖能力、迁移细胞数和侵袭细胞数显著降低($P<0.05$,图2)。

2.3 miR-342-3p过表达对ccRCC移植瘤模型成瘤的影响

见图3。与miR-NC组比较,miR-342-3p组可明显促进肿瘤生长(图3A)。观察14 d处死裸鼠,与miR-NC组相

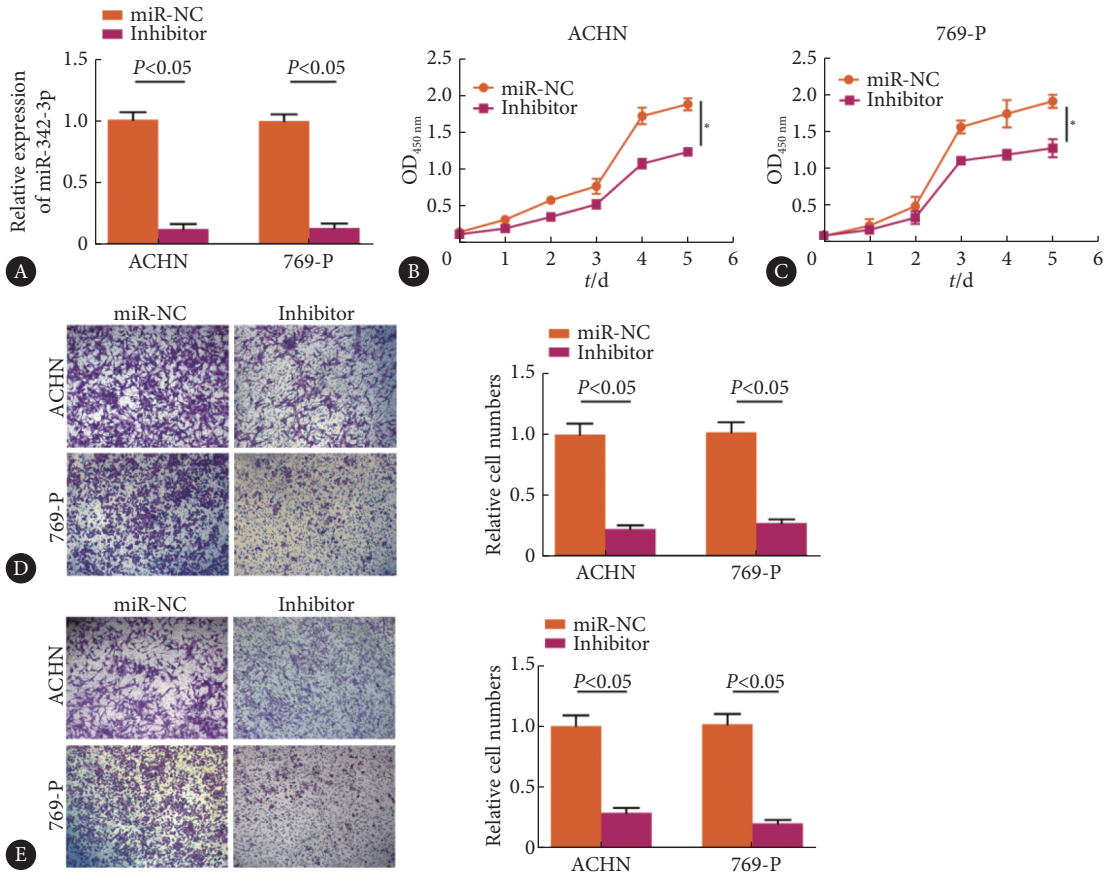

图 2 miR-342-3p低表达对ccRCC细胞增殖、迁移和侵袭的影响

Fig 2 Effects of the low-expression miR-342-3p on the proliferation, migration, and invasion of ccRCC cells

A, The level of miR-342-3p detected by RT-qPCR; B and C, effects of the low-expression miR-342-3p on the proliferation of ccRCC cells; D, effects of the low-expression miR-342-3p on the migration of ccRCC cells; E, effects of the low expression miR-342-3p on the invasion of ccRCC cells. n=3, * P<0.05, at Day 5.

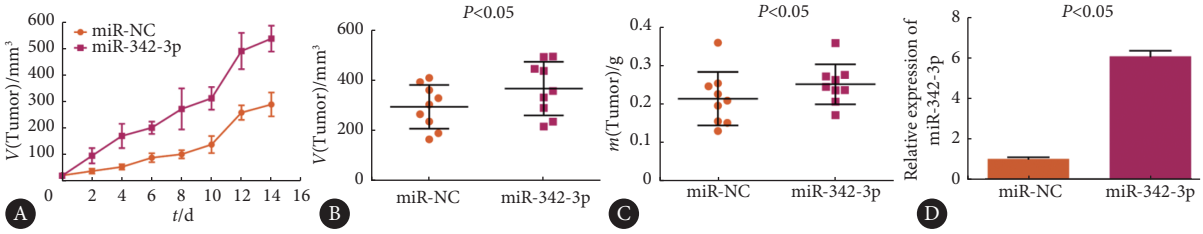

图 3 miR-342-3p过表达对ccRCC移植瘤模型成瘤的影响

Fig 3 Effects of miR-342-3p overexpression on tumor formation in the ccRCC xenograft model

A, Tumor growth curve; B, tumor volume on day 14; C, tumor mass on day 14; D, the relative expression level of miR-342-3p detected by RT-qPCR on day 14. n=9.

比较, miR-342-3p组肿瘤组织体积及质量明显增加($P<0.05$, 图3B ~ 3C), 肿瘤组织中miR-342-3p表达水平明显增加, *PPM1E* mRNA表达水平明显降低($P<0.05$, 图3D)。

2.4　miR-342-3p与*PPM1E*的靶向关系

下载GSE66271、GSE66270数据集分析下调基因, 再与TCGA中与转移相关的基因数据矩阵及starBase预测miR-342-3p的靶基因进行交集分析, 结果显示有7个交集基因, 分别是*PPM1E*、*ADRB1*、*ERBB4*、*FXYD3*、*GRHL2*、*NRK*、*PCP4*。进一步分析这7个基因与ccRCC恶性临床表型的关系(图4A)。其中, *PPM1E*在ccRCC组织和癌旁正常组织、不同M分期、N分期、G分期及复发情况患者中存在差异表达($P<0.05$, 图4B ~ 4F)。故选择*PPM1E*进一步验证其与miR-342-3p靶向关系, 图4G为二者结合位点信息, 其与miR-NC组比较, 共转染*PPM1E*-WT-3'-UTR萤

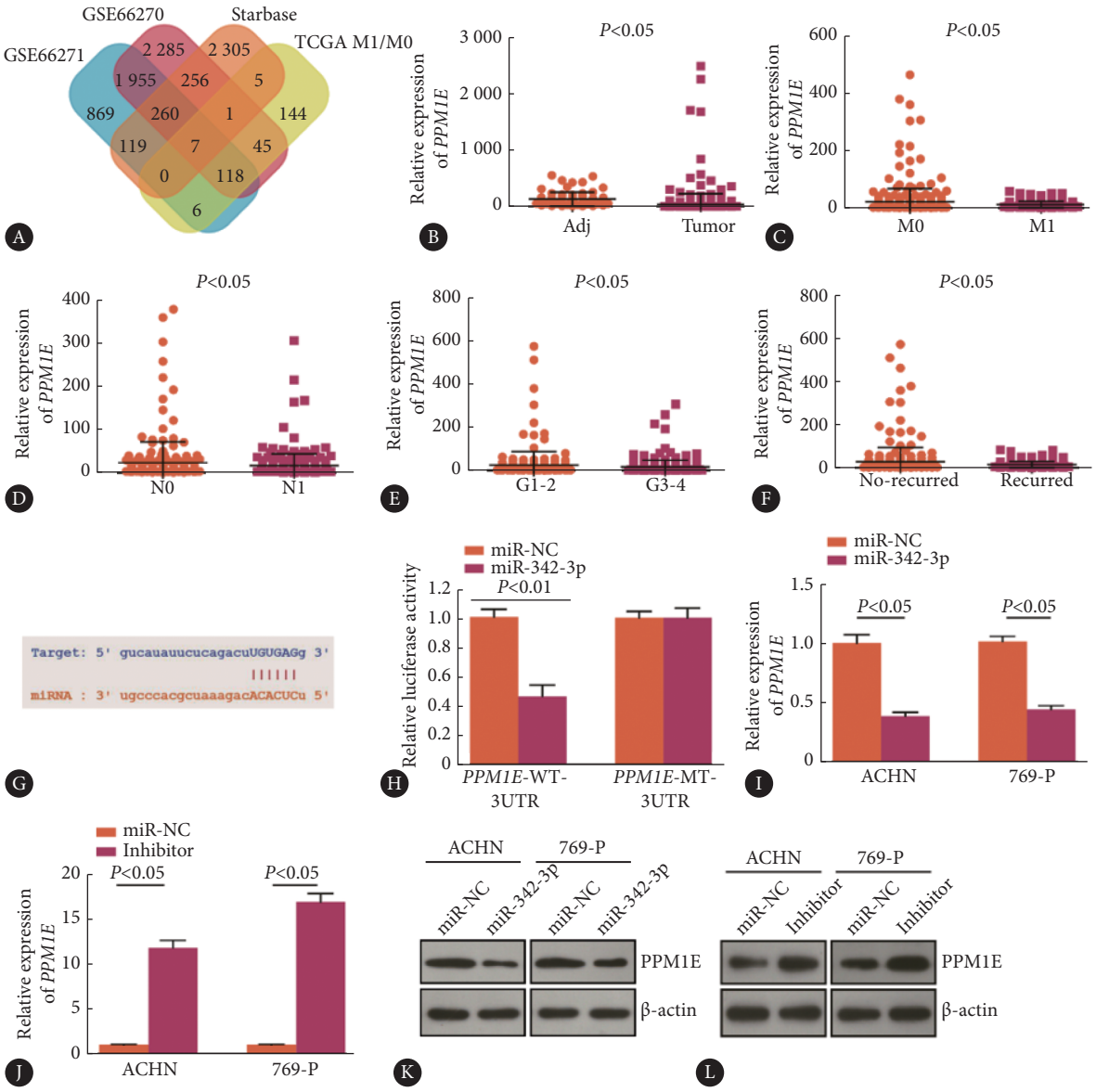

图 4　miR-342-3p与PPM1E靶向关系

Fig 4　Targeted relationship between miR-342-3p and PPM1E

A, Intersection analysis of GSE66271, GSE66270, and TCGA was conducted with gene data matrixes related to metastasis and starBase (sRNA target Base) to predict miR-342-3p target genes. B, *PPM1E* expression in tumor group (n=532) and Adj group (n=72). C, Comparison of *PPM1E* expression in patients with tumors of M0 stage (n=415) and M1 stage (n=106). D, Comparison of PPM1E expression in patients with tumors of N0 stage (n=237) and N1 stage (n=287). E, Comparison of *PPM1E* expression in patients with tumors of grades G1-2 (n=239) and grades G3-4 (n=281). F, Comparison of *PPM1E* expression in patients of no-recurrence statuse (n=303) and recurrence statuse (n=124). G, Possible binding sites between miR-342-3p and *PPM1E* mRNA at 3'-UTR site; H, targeted relationship between miR-342-3p and *PPM1E* verified by dual luciferase reporter genes (n=3). I-J, Effects of miR-342-3p low expression/overexpression on *PPM1E* mRNA (n=3). K-L, Effects of miR-342-3p low expression/overexpression on PPM1E protein.

光素酶报告基因载体与miR-342-3p mimic的细胞萤光素酶活性显著降低($P<0.05$, 图4H)。*PPM1E* mRNA的表达量, 无论在ACHN细胞中还是在769-P细胞中, 均是miR-NC组低于inhibitor组, 差异有统计学意义($P<0.05$, 图4I)。图4K, miR-NC组和miR-342-3p组的PPM1E蛋白相对表达量, ACHN细胞中分别为0.94±0.12、0.29±0.03, 769-P细胞中分别为1.02±0.13、0.31±0.06。miR-NC组PPM1E蛋白相对表达量均高于miR-342-3p组, 差异有统计学意义($P<0.05$)。图4L, miR-NC组和inhibitor组的PPM1E蛋白相对表达量, ACHN细胞中分别为0.97±0.08、1.76±0.28, 769-P细胞中分别为1.04±0.05、1.75±0.12。miR-NC组的PPM1E蛋白相对表达量均低于inhibitor组, 差异有统计学意义($P<0.05$)。

2.5 miR-342-3p过表达靶向PPM1E对ccRCC细胞增殖、迁移和侵袭的影响

见图5。图5A, miR-NC组、miR-342-3p组和miR-342-3p+PPM1E组PPM1E蛋白相对表达量, 在ACHN细胞中, 分别为0.98±0.16、0.42±0.07和0.91±0.12, 在769-P细胞中, 分别为0.99±0.05、0.57±0.04和0.95±0.27。与miR-NC组相比较, miR-342-3p组PPM1E蛋白表达降低, 细胞增殖能力、迁移细胞数和侵袭细胞数明显升高($P<0.05$); 与miR-342-3p组相比较, miR-342-3p+PPM1E组PPM1E蛋白表达升高, 细胞增殖能力、迁移细胞数和侵袭细胞数明显降低($P<0.05$)。

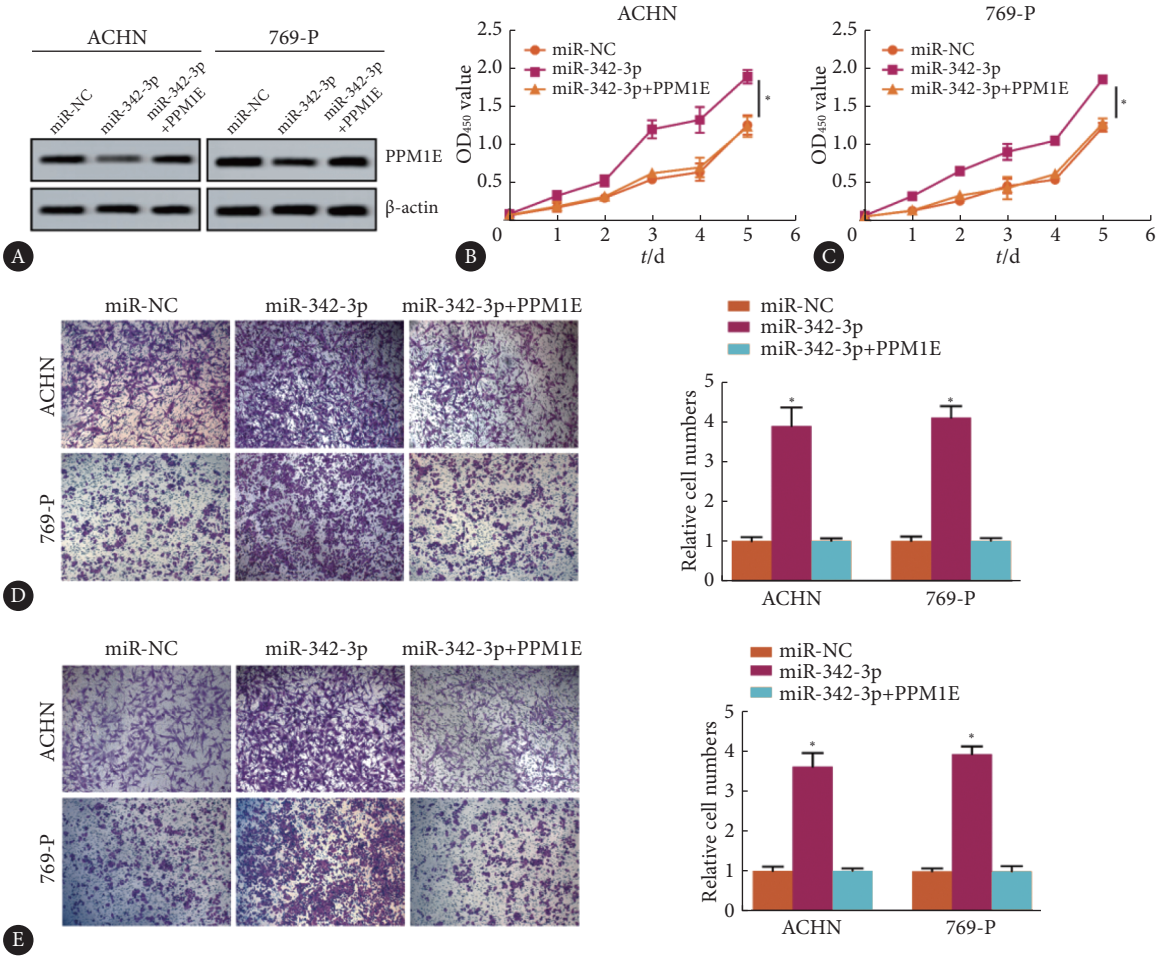

图 5 miR-342-3p过表达靶向PPM1E对ccRCC细胞增殖、迁移和侵袭的影响

Fig 5 Effects of miR-342-3p overexpression on the proliferation, migration, and invasion of ccRCC cells by targeting PPM1E

A, The expression of PPM1E protein detected by Western blot; B and C, the effects of miR-342-3p overexpression on the proliferation of ccRCC cells by targeting PPM1E; D, the effects of miR-342-3p overexpression on the migration of ccRCC cells by targeting PPM1E; E, the effects of miR-342-3p overexpression on the invasion of ccRCC cells by targeting PPM1E. $n=3$. $^*P<0.05$.

3 讨论

miRNAs几乎在所有肿瘤细胞中均存在异常表达, 这种失调表达可能参与肿瘤的发生发展, 基于miRNAs的靶向治疗已成为各类恶性肿瘤研究的热点[8-9]。研究显示, miR-342-3p在多种癌症中表达下调, 如肺癌[10]、宫颈

癌[11]、甲状腺癌[12]等, 但在肝癌中发现了miR-342-3p表达上调[13], 提示miR-342-3p在恶性肿瘤中具有双重作用。本研究通过分析GSE12105和GSE23085数据矩阵差异基因信息, 发现2个在上述GEO数据中共同上调表达的miRNA, 结合TCGA_ccRCC数据和临床信息, 表明miR-342-3p可能与患者T分期、G分期、预后情况密切相关, 生存分析显示, miR-342-3p高表达患者总生存时间更短, 且miR-342-3p可作为区分ccRCC的诊断标志物, 这与张霞军等[14]研究基本一致。但miR-342-3p在ccRCC中的具体作用机制尚不清楚。

研究发现, miR-342-3p可靶向下游基因, 影响肿瘤细胞恶性行为。CUI等[15]发现miR-342-3p下调可预测鼻咽癌患者的不良预后, 还可靶向抑制FOXQ1表达, 抑制鼻咽癌的生长和侵袭。WANG等[16]检测发现, miR-342-3p在口腔鳞状细胞癌细胞系和临床标本中表达下调, 通过靶向LASP1发挥抑癌作用。本研究构建稳定低表达miR-342-3p的人ccRCC细胞ACHN、769-P, 发现miR-342-3p低表达可抑制ccRCC细胞体外的增殖、迁移和侵袭活性, Balb/c裸鼠体内接种miR-342-3p过表达的ACHN细胞后, 可明显促进肿瘤的生长。上述结果表明, miR-342-3p在ccRCC中作为促癌因子, 可促进ccRCC细胞增殖、迁移和侵袭能力。

研究发现, miRNAs主要通过靶向结合靶基因mRNA的3'-UTR端, 抑制其表达来发挥生物学功能[17-18]。本研究通过下载GSE66271、GSE66270数据集分析下调基因, 再与TCGA中与转移相关的基因数据矩阵及starBase预测miR-342-3p的靶基因进行交集分析, 结果显示存在7个交集基因。进一步分析与TCGA数据集ccRCC恶性临床表型相关基因, 结果显示, PPM1E在ccRCC组织中表达下调, 且在不同M分期、N分期、G分期及复发情况患者中存在差异表达, 故选择PPM1E进一步验证其与miR-342-3p靶向关系。后续双萤光素酶报告基因检测、实时荧光定量PCR和蛋白印迹实验证明, miR-342-3p过表达可降低PPM1E表达。另外, PPM1E可逆转miR-342-3p过表达对ccRCC细胞增殖、迁移和侵袭的促进作用, 表明miR-342-3p过表达促进ccRCC细胞增殖、迁移和侵袭, 可能是通过负向调节PPM1E来实现的。PPM1E属于蛋白磷酸酯酶, 蛋白激酶和蛋白磷酸酯酶是催化可逆性蛋白磷酸化和去磷酸化的关键酶系, 对蛋白活性状态的修饰有重要调控作用[19], 但有关蛋白磷酸酯酶方面的研究仍存在不足, PPM1E在肿瘤细胞中的调控作用可能与AMPK活性有关[20], 但其在ccRCC中的具体作用途径仍有待深入分析。

综上所述, miR-342-3p过表达可靶向抑制PPM1E表达, 从而促进ccRCC细胞增殖、迁移和侵袭。本研究为ccRCC进展的分子机制提供了新思路, 进一步表明miR-342-3p可能作为ccRCC新型的生物标记物和治疗靶点。

*　　*　　*

作者贡献声明　王荦楠负责论文构思、正式分析、经费获取和审读与编辑写作, 李卓然负责研究方法、研究项目管理、提供资源、软件、监督指导和初稿写作, 吴洁清负责验证和可视化, 谢晋玲负责数据审编和调查研究。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。

Author Contribution　Wang Luonan is responsible for conceptualization, formal analysis, funding acquisition, and writing--review and editing. Li Zhuoran is responsible for methodology, project administration, resources, software, supervision, and writing--original draft. Wu Jieqing is responsible for validation and visualization. Xie Jinling is responsible for data curation and investigation. 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.

利益冲突　所有作者均声明不存在利益冲突

Declaration of Conflicting Interests　All authors declare no competing interests.

参　考　文　献

[1] 卢文卿, 金悦, 刘云鹏, 等. 基于SIRTUINs和临床病理特征的肾透明细胞癌预后评估模型建立及验证. 山东医药, 2020, 1169(11): 6–10. doi: 10.3969/j.issn.1002-266X.2020.11.001.
LU W Q, JIN Y, LIU Y P, *et al*. Establishment and identification of prognostic assessment model for clear-cell renal-cell carcinoma based on SIRTUINs family and clinicopathologic features. Shandong Med J, 2020, 1169(11): 6–10. doi: 10.3969/j.issn.1002-266X.2020.11.001.

[2] LINEHAN W M, RICKETTS C J. The Cancer Genome Atlas of renal cell carcinoma: findings and clinical implications. Nat Rev Urol, 2019, 16(9): 539–552. doi: 10.1038/s41585-019-0211-5.

[3] 胡金芳, 陈思羽, 蔡嘉怡. miRNA对肿瘤微环境中免疫细胞的调控作用. 医学综述, 2020, 26(11): 2156–2163. doi: 10.3969/j.issn.1006-2084.2020.11.014.
HU J F, CHEN S Y, CAI J Y. Regulatory role of microRNAs on immune cells in tumor microenvironment. Med Recapitulate, 2020, 26(11): 2156–2163. doi: 10.3969/j.issn.1006-2084.2020.11.014.

[4] WANG C, ZHANG W, XING S, *et al*. MiR-342-3p inhibits cell migration and invasion through suppressing forkhead box protein Q1 in ovarian carcinoma. Anticancer Drugs, 2019, 30(9): 917–924. doi: 10.1097/CAD.0000000000000801.

[5] 金浩, 余佳, 王梓瑜, 等. 槐角黄酮通过调控LncRNA FBXL19-AS1/miR-342-3p通路抑制肝癌细胞增殖, 迁移及侵袭的机制研究. 中国中药杂志, 2020, 45(18): 162–169. doi: 10.3969/j.issn.1005-9202.2020.20.038.
JIN H, YU J, WANG Z Y, *et al*. Mechanism of flavonoids of Sophorae Fructus in inhibiting proliferation, migration and invasion of

hepatocellular carcinoma cells by regulating LncRNA FBXL19-AS1/miR-342-3p pathway. Chin J Chin Mater Med, 2020, 45(18): 162–169. doi: 10.3969/j.issn.1005-9202.2020.20.038.

[6] CHEN M B, LIU Y Y, CHENG L B, *et al*. AMPKα phosphatase Ppm1E upregulation in human gastric cancer is required for cell proliferation. Oncotarget, 2017, 8(19): 31288–31296. doi: 10.18632/oncotarget.16126.

[7] LI Z, GUO X, TANG L, *et al*. Methylation analysis of plasma cell-free DNA for breast cancer early detection using bisulfite next-generation sequencing. Tumour Biol, 2016, 37(10): 13111–13119. doi: 10.1007/s13277-016-5190-z.

[8] 何静宇, 周雪琴, 王文涛. miRNA-3679抑制下游ZADH2-靶基因促进肝癌细胞增殖的机制研究. 四川大学学报(医学版), 2022, 53(5): 744–751. doi: 10.12182/20220960505.

HE J Y, ZHOU X Q, WANG W T. Mechanism of miRNA-3679 inhibiting downstream ZADH2-target genes to promote hepatocellular carcinoma cell proliferation. J Sichuan Univ (Med Sci), 2022, 53(5): 744–751. doi: 10.12182/20220960505.

[9] 樊松强, 张红森, 汤尧. 外泌体介导miR-199a-3p靶向*ARPC2*基因对肾癌细胞增殖, 迁移和侵袭的影响. 中国老年学杂志, 2022, 42(4): 934–939. doi: 10.3969/j.issn.1005-9202.2022.04.048.

FAN S Q, ZHANG H S, TANG Y. Effects of exosome-mediated miR-199a-3p on proliferation, migration and invasion of renal carcinoma cells by targeting *ARPC2* gene. Chin J Gerontol, 2022, 42(4): 934–939. doi: 10.3969/j.issn.1005-9202.2022.04.048.

[10] 朱亚蕊, 时松和, 李雪. 慢病毒载体介导的miR-342-3p对非小细胞肺癌细胞增殖, 侵袭迁移的影响研究. 临床肺科杂志, 2021, 26(10): 1565–1568. doi: 10.3969/j.issn.1009-6663.2021.10.023.

ZHU Y R, SHI S H, LI X. Effect of miR-342-3p mediated by lentiviral vector on the proliferation, invasion and migration of non-small cell lung cancer cells. J Clin Pulmon Med, 2021, 26(10): 1565–1568. doi: 10.3969/j.issn.1009-6663.2021.10.023.

[11] 林佳芝,宁舒婷,邓金金,等. 紫花牡荆素通过上调miR-342-3p抑制宫颈癌HeLa细胞糖酵解. 暨南大学学报(自然科学与医学版), 2023,44(5):460-469. doi: 10.11778/j.jdxb.20230085.

LIN J Z, NING S T, DENG J J, *et al*. Casticin inhibited glycolysis by upregulation of miR-342-3p expression in cervical cancer HeLa cells. J Jinan Univ (Nat Sci Med Edn), 2023,44(5):460-469. doi: 10.11778/j.jdxb.20230085.

[12] 骆锴冉, 谢克举, 孟凡波, 等. miR-342-3p通过靶向Bcl-2上调TP53蛋白表达抑制甲状腺癌细胞增殖. 中华内分泌外科杂志, 2020, 14(5): 393–398. doi: 10.3760/cma.j.cn.115807-20200812-00244.

LUO K R, XIE K J, MENG F B, *et al*. miR-342-3p regulates thyroid cancer cell proliferation, migration and invasion by targeting Bcl-2. Chin J Endocr Surg, 2020, 14(5): 393–398. doi: 10.3760/cma.j.cn.115807-20200812-00244.

[13] KOMOLL R M, HU Q, OLAREWAJU O, *et al*. MicroRNA-342-3p is a potent tumour suppressor in hepatocellular carcinoma. J Hepatol, 2020, 74(1): 122–134. doi: 10.1016/j.jhep.2020.07.039.

[14] 张霞军, 胡孟谋, 袁红萍, 等. 基于生物信息学分析肾透明细胞癌组织中差异过表达miRNAs及其临床意义. 生物技术, 2020, 180(5): 47–54. doi: 10.16519/j.cnki.1004-311x.2020.05.0073.

ZHANG X J, HU M M, YUAN H P, *et al*. Bioinformatics analysis of up-regulated miRNAs in clear cell renal cell carcinoma and their clinical significance. Biotechnology, 2020, 180(5): 47–54. doi: 10.16519/j.cnki.1004-311x.2020.05.0073.

[15] CUI Z, ZHAO Y. MicroRNA-342-3p targets FOXQ1 to suppress the aggressive phenotype of nasopharyngeal carcinoma cells. BMC Cancer, 2019, 19(1): 104. doi: 10.1186/s12885-018-5225-5.

[16] WANG Y, GUO X, WANG L, *et al*. miR-342-3p inhibits acute myeloid leukemia progression by targeting SOX12. Oxid Med Cell Longev, 2022, 2022:1275141. doi: 10.1155/2022/1275141.

[17] SOUSA M, DOLICKA D, GJORGJIEVA M, *et al*. Deciphering miRNAs' action through miRNA editing. Int J Mol Sci, 2019, 20(24): 6249. doi: 10.3390/ijms20246249.

[18] LIU X L, PAN W G, LI K L, *et al*. MiR-1293 suppresses tumor malignancy by targeting hydrocyanic oxidase 2: therapeutic potential of a miR-1293/hydrocyanic oxidase 2 axis in renal cell carcinoma. Cancer Biother Radiopharm, 2020, 35(5): 377–386. doi: 10.1089/cbr.2019.2957.

[19] RUVOLO P P. Role of protein phosphatases in the cancer microenvironment. Biochim Biophys Acta Mol Cell Res, 2019, 1866(1): 144–152. doi: 10.1016/j.bbamcr.2018.07.006.

[20] LI Z W, ZHU Y R, ZHOU X Z, *et al*. MicroRNA-135b expression silences Ppm1e to provoke AMPK activation and inhibit osteoblastoma cell proliferation. Oncotarget, 2017, 8(16): 26424–26433. doi: 10.18632/oncotarget.15477.

（2023 – 06 – 12收稿, 2024 – 04 – 30修回）

编辑 何学令