·论 著·

tRF-Pro-CGG对小鼠胰腺癌细胞生物学行为的影响及其分子机制

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［**摘要**］ **背景与目的：**tRNA衍生的片段（tRNA-derived fragments，tRF）是一类长度为14～30 nt的小分子非编码RNA，其影响着恶性肿瘤的发展进程。本研究旨在探讨tRF-Pro-CGG对小鼠胰腺癌细胞生物学行为的影响及其可能的分子机制。**方法：**采用实时荧光定量聚合酶链反应（real-time fluorescence quantitative polymerase chain reaction，RTFQ-PCR）检测tRF-Pro-CGG在小鼠胰腺癌细胞系pan02、LTPA，人胰腺癌细胞系Capan-2和正常胰腺细胞HPDE6-C7中的表达水平。通过慢病毒转染技术过表达pan02细胞及敲低LTPA细胞中tRF-Pro-CGG的表达，采用RTFQ-PCR和蛋白质印迹法（Western blot）检测过表达和敲低效果。采用细胞计数试剂盒-8（cell counting kit-8，CCK-8）检测细胞增殖情况。采用transwell实验检测细胞迁移和侵袭能力。采用动物模型检测tRF-Pro-CGG对胰腺癌裸鼠移植瘤生长和转移的影响。采用H-E染色观察移植瘤的组织病理学结构。采用Western blot检测移植瘤组织中Ki-67增殖指数、转移相关蛋白E-钙黏蛋白（E-cadherin）、波形蛋白（vimentin）的表达及磷脂酰肌醇3-激酶（phosphoinositide 3-kinase，PI3K）/蛋白激酶B（protein kinase B，AKT）信号转导通路蛋白的表达及磷酸化情况。**结果：**小鼠胰腺癌细胞系pan02中tRF-Pro-CGG表达最低，在pan02细胞中转染tRF-Pro-CGG mimics后，tRF-Pro-CGG的mRNA和蛋白表达水平均显著升高（$P<0.01$），细胞增殖能力显著降低（$P<0.01$），细胞迁移（$P<0.001$）和侵袭能力（$P<0.001$）显著降低，裸鼠移植瘤体积（$P<0.01$）和重量（$P<0.001$）均显著降低，裸鼠移植瘤组织中出现明显坏死和凋亡细胞；裸鼠移植瘤组织中Ki-67增殖指数和vimentin的表达显著降低（$P<0.001$），而E-cadherin的表达显著升高（$P<0.001$），PI3K、P-PI3K、AKT和P-AKT的表达显著降低（$P<0.001$），胰腺癌肝转移例数差异无统计学意义（$P>0.05$）。小鼠胰腺癌细胞系LTPA中tRF-Pro-CGG表达最高，在LTPA细胞中转染tRF-Pro-CGG inhibitor后，tRF-Pro-CGG的mRNA和蛋白表达水平显著降低（$P<0.01$），细胞增殖能力显著升高（$P<0.01$），细胞迁移（$P<0.001$）和侵袭能力（$P<0.001$）显著升高，裸鼠移植瘤体积（$P<0.01$）和重量（$P<0.01$）均显著升高，裸鼠移植瘤组织中出现少量的坏死及凋亡细胞；裸鼠移植瘤组织中Ki-67和vimentin的表达显著升高（$P<0.001$），而E-cadherin的表达显著降低（$P<0.001$），PI3K、P-PI3K、AKT和P-AKT的表达显著升高（$P<0.001$），胰腺癌肝转移例数差异无统计学意义（$P>0.05$）。**结论：**过表达tRF-Pro-CGG可以抑制小鼠胰腺癌细胞增殖、迁移和侵袭，抑制胰腺癌裸鼠移植瘤的生长，下调Ki-67增殖指数、vimentin的表达水平和PI3K/AKT磷酸化水平。tRF-Pro-CGG可能通过调节PI3K/AKT信号转导通路抑制胰腺癌的发生、发展。

［**关键词**］胰腺癌；tRNA衍生的片段-Pro-CGG；增殖；迁移；侵袭；裸鼠移植瘤模型
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Effect of tRF-Pro-CGG on the biological behavior of mouse pancreatic cancer cells and its molecular mechanism

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［**Abstract**］ **Background and purpose:** tRNA-derived fragments (tRF) are a kind of short non-coding RNA (14-30 nt) that influences the course of cancer. This study aimed to investigate the molecular pathways that might underlie the effects of tRF-Pro-CGG on the biological behavior of mouse pancreatic cancer cells. **Methods:** Real-time fluorescence quantitative polymerase chain reaction (RTFQ-PCR) was used to assess the expression of tRF-Pro-CGG in mouse pancreatic cancer cell lines pan02 and LTPA, human pancreatic cancer cell line Capan-2, and normal pancreatic cells HPDE6-C7. tRF-Pro-CGG overexpression in pan02 cells and LTPA cell suppression were achieved through lentiviral transfection, and RTFQ-PCR and Western blot were used to determine overexpression and knockdown effects. Cell counting kit-8 (CCK-8) was used to detect cell proliferation. Transwell assays were used to detect cell migration and invasion ability. The effect of tRF-Pro-CGG on the growth and metastasis of pancreatic cancer transplantation tumors in nude mice model was investigated. H-E staining was used to observe the histopathological structure of transplantation tumors. Western blot was used to detect the expression and phosphorylation of proliferation-related protein Ki-67 and metastasis-related proteins. Western blot was used to assess the expressions of cadherin, vimentin, phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway protein and phosphorylation in transplanted tumor tissues. **Results:** tRF-Pro-CGG expression was lowest in the mouse pancreatic cancer cell line pan02. Both mRNA and protein expression levels of tRF-Pro-CGG were significantly increased ($P<0.01$) after transfection of tRF-Pro-CGG mimics in pan02 cells, and cell proliferation ability ($P<0.01$), cell migration ($P<0.001$) and invasion ability ($P<0.001$) were significantly reduced. A significant decrease in the volume ($P<0.01$) and weight ($P<0.001$) of transplanted tumors in nude mice was observed, and significant necrotic and apoptotic cells in transplanted tumor were identified. In transplanted tumor tissues of nude mice, the Ki-67 proliferatien index and expression of vimentin were significantly decreased ($P<0.001$), while E-cadherin was increased ($P<0.001$). The expressions of PI3K, P-PI3K, AKT and P-AKT were significantly decreased ($P<0.001$). There was no significant difference in the number of liver metastases from pancreatic cancer ($P>0.05$). The mouse pancreatic cancer cell line LTPA had the greatest level of tRF-Pro-CGG expression. The mRNA and protein expression levels of tRF-Pro-CGG were significantly reduced ($P<0.01$) after transfection of tRF-Pro-CGG inhibitor in LTPA cells. The proliferation ability of cells was significantly increased ($P<0.01$), the migration of cells ($P<0.001$) and invasive ability ($P<0.001$) were significantly increased. The volume ($P<0.01$) and weight ($P<0.01$) of transplanted tumors in nude mice were significantly increased, and a limited proportion of necrotic and apoptotic cells were seen in nude mice tumor tissues implanted. In the transplanted tumor tissues of nude mice, the Ki-67 proliferation index and expression of vimentin were significantly increased ($P<0.001$), while E-cadherin was decreased ($P<0.001$). The expressions of PI3K, P-PI3K, AKT, and P-AKT were significantly increased ($P<0.001$). There was no difference in the number of liver metastases from pancreatic cancer ($P>0.05$). **Conclusion:** Overexpression of tRF-Pro-CGG reduced pancreatic cancer cell proliferation, migration and invasion in mice, slowed the formation of pancreatic cancer transplanted tumors in nude mice, and decreased Ki-67 proliferation index and expression of vimentin and PI3K/AKT phosphorylation levels. The PI3K/AKT signaling pathway may be regulated by tRF-Pro-CGG, which may suppress the development of pancreatic cancer.

［**Key words**］ Pancreatic cancer; tRNA-derived fragments-Pro-CGG; Proliferation; Migration; Invasion; Tumor transplantation model in nude mice

胰腺癌因其发病隐匿，早期缺乏诊断方法，确诊时多为中晚期，5年生存率为7%～8%[1]。在临床上，胰腺癌的治疗方法有手术治疗、放疗、化疗、靶向治疗及免疫治疗等，然而其复发转移率仍较高，预后极差[2]。寻求新的肿瘤标志物以早期诊断及治疗胰腺癌有重大意义。tRNA衍生的片段（tRNA-derived fragments，tRF）来源于tRNA的小分子非编码RNA（small non-coding RNA，sncRNA），由前体或成熟tRNA片段衍生而来，其长度为14～30 nt[3]。tRF在乳腺癌、前列腺癌、结直肠癌及肺癌等肿瘤中发挥重要作用，然而在胰腺癌中的表达及相关作用机制尚不明确。本课题组的前期研究[4]经tRF、tRNA衍生的应激诱导RNA（tRNA-derived stress-induced RNAs，tiRNA）测序及标本组织实时荧光定量聚合酶链反应（real-time fluorescence quantitative polymerase chain reaction，RTFQ-PCR）证实tRF-Pro-CGG在胰腺癌和正常胰腺组织中差异表达，人胰腺癌组织中tRF-Pro-CGG的表达比正常胰腺组织低，提示tRF-Pro-CGG在胰腺癌中表达不足，与临床生存期短和预后差相关[5]。本研究旨在探讨*tRF-Pro-CGG*基因在小鼠

胰腺癌细胞中的表达及其对相关生物学行为的影响，并探讨其可能的分子机制。

1 材料和方法

1.1 材料

小鼠胰腺癌细胞系pan02、人胰腺癌细胞系Capan-2和正常胰腺细胞HPDE6-C购自中国科学院上海生命科学研究院细胞资源中心，小鼠胰腺癌细胞系LTPA购自上海弘顺生物科技有限公司，胎牛血清（fetal bovine serum，FBS）、DMEM培养基、胰蛋白酶、青链霉素购自美国Gibco公司，tRF-Pro-CGG mimics、NC mimics、tRF-Pro-CGG inhibitor、NC inhibitor质粒、抗tRF-Pro-CGG及riboFECTTMCP转染试剂购自广州锐博生物技术有限公司，RTFQ-PCR试剂盒购自日本Takara公司，细胞计数试剂盒（cell counting kit-8，CCK-8）购自美国MCE公司，Matrigel购自美国BD公司，免疫印迹化学发光试剂购自美国Millipore公司，β-actin抗体、抗Ki-67抗体、抗vimentin、抗E-cadherin、抗PI3K、抗P-PI3K、抗AKT及抗P-AKT购自英国Abcam公司，BALB/c裸鼠购自杭州子源实验动物科技有限公司。

1.2 方法

1.2.1 细胞培养和转染

上述细胞在37 ℃、CO_2体积分数为5%的DMEM培养基中培养，该培养基含有10%的FBS、100 U/mL链霉素和100 U/mL青霉素。将处于对数生长期的细胞以每孔2.5×10^5个细胞/mL的密度加入到6孔板中，用完全培养基培养1 d，直到密度达到80%。根据Lipofectamine^{TM}3000的使用说明书，用tRF-Pro-GG mimics及inhibitor转染相关细胞后培养24 h，进行后续实验。

1.2.2 RTFQ-PCR实验

利用TRIzol试剂（美国ThermoFisher公司）分离细胞中的总RNA，通过反转录总RNA和扩增试剂盒制备cDNA模板，并进行RTFQ-PCR扩增，扩增条件为：95 ℃ 5 min；95 ℃ 15 s，58 ℃ 30 s，72 ℃ 30 s，共40个循环。本研究使用的引物购自广州锐博生物技术有限公司，*U6*被用作内部参考。*tRF-Pro-CGG*引物的正义链为5′-GGCTCGTTGGTCTAGGGGTATG-3′，反义链为5′-ACGTGTGCTCTTCCGATCTGAA-3′；U6引物的正义链为5′-GCTTCGGCAGCACATATACTAAAAT-3′，反义链为5′-CGCTTCACGAATTTGCGTGTCAT-3′。利用$2^{-\Delta\Delta Ct}$计算tRF-Pro-CGG的平均相对表达水平。

1.2.3 CCK-8实验

将对数生长期的细胞以1×10^4个细胞/孔均匀铺设于96孔板中，每孔体积为100 μL，细胞附壁后分别于第0、24、48、72和96 h加入10 μL CCK-8试剂，外围孔填充磷酸缓冲盐溶液（phosphate-buffered saline，PBS），将96孔板置于37 ℃、CO_2体积分数为5%的培养箱中温育2 h，测量450 nm处细胞的吸光度（*D*）值。

1.2.4 Transwell迁移和侵袭实验

将处于对数生长期的细胞饥饿6 h，用无血清培养基将50 mg/L的matrigel按1∶8的比例稀释，使用前与培养基混合，用胰蛋白酶消化细胞后用含有10%FBS的完全培养基终止消化，细胞以10 000 r/min离心5 min后弃去上清液，PBS洗涤2次后用无血清培养基重新悬浮。细胞计数后在含有基质和不含基质的上腔室中分别加入5×10^4个细胞，体积均为500 μL。将腔室放入24孔板中，并在下腔室中加入700 μL含有10%FBS的完全培养基，细胞在37 ℃、CO_2体积分数为5%的条件下培养24 h，随后取出腔体放入新的孔中，并用PBS清洗2次，用4%多聚甲醛溶液固定15 min后，用800 μL 0.5%结晶紫染色液染色20 min，干燥后在显微镜下对细胞进行计数并拍照。

1.2.5 动物实验

选取5周龄雌性BALB/c裸鼠随机分为4组（每组9只），将过表达tRF-Pro-CGG（tRF-Pro-CGG mimics组和NC mimics组）的pan02细胞及敲低tRF-Pro-CGG（tRF-Pro-CGG inhibitor组和NC inhibitor组）的LTPA细胞分别经皮下注射到裸鼠体内，每只裸鼠注射浓度为5×10^6个细胞/mL的0.2 mL单细

胞悬液，建立胰腺癌裸鼠皮下移植瘤模型。利用动物活体成像系统观察并记录肿瘤生长情况，取下瘤体后称重，将瘤体在4%多聚甲醛溶液中固定后常规做H-E染色。建立裸鼠胰腺癌肝转移模型，随机分为4组（每组5只），将过表达tRF-Pro-CGG（tRF-Pro-CGG mimics组和NC mimics组）的pan02细胞及敲低tRF-Pro-CGG（tRF-Pro-CGG inhibitor组和NC inhibitor组）的LTPA细胞分别经尾静脉注射到裸鼠体内，每只裸鼠注射浓度为2×10^6个细胞/mL的0.2 mL单细胞悬液，每隔4 d经尾静脉追加上述各组细胞，10周后处死解剖裸鼠并观察胰腺癌肝脏转移瘤的结节个数。

1.2.6 蛋白质印迹法（Western blot）实验

收集各组稳定转染tRF-Pro-CGG的细胞或液氮研磨肿瘤组织，添加裂解液（美国Sigma-Aldrich公司）后从细胞或组织中提取总蛋白，用二辛可宁酸（bicinchoninic acid，BCA）试剂盒检测蛋白浓度，用十二烷基硫酸钠聚丙烯酰胺凝胶电泳（sodium dodecylsulphate polyacrylamide gel electrophoresis，SDS-PAGE）将蛋白转移到PVDF膜上，用5%脱脂牛奶密封4 h，加入抗tRF-Pro-CGG、Ki-67、抗vimentin、抗E-cadherin、抗PI3K、P-PI3K、抗AKT及P-AKT的一抗4 ℃温育蛋白过夜，含有吐温-20三乙醇胺缓冲盐溶液（tris-buffered saline Tween，TBST）洗涤后加入二抗，在常温下温育1 h，最后加入免疫印迹化学发光试剂后凝胶成像曝光，以β-肌动蛋白为内部对照，用ImageJ软件分析目标蛋白的灰色值。

1.3 统计学处理

服从正态分布的计量资料采用$\bar{x}\pm s$表示，两组比较采用独立样本t检验，多组间比较采用单因素方差分析。计数资料两组比较采用Fisher确切概率法。$P<0.05$为差异有统计学意义。

2 结 果

2.1 tRF–Pro–CGG在胰腺癌细胞系中的表达

RTFQ-PCR结果显示，tRF-Pro-CGG在小鼠胰腺癌细胞系pan02、LTPA和人胰腺癌细胞系Capan-2中的表达低于在正常胰腺细胞HPDE6-C中的表达，在pan02细胞中的表达最低，在LTPA细胞中的表达相对较高，故选用pan02细胞和LTPA细胞进行tRF-Pro-CGG的过表达及敲低实验（图1）。

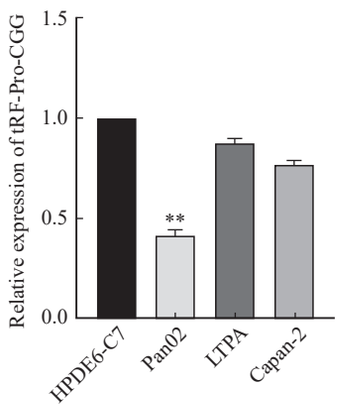

图1 tRF–Pro–CGG在小鼠胰腺癌细胞系pan02、LTPA及人胰腺癌细胞系Capan–2中的表达

Fig. 1 tRF-Pro-CGG expression in mouse pancreatic cancer cell lines pan02 and LTPA, as well as human pancreatic cancer cell line Capan-2

**: $P<0.01$, compared with HPDE6-C cell line.

2.2 tRF–Pro–CGG稳定过表达及敲低细胞系的构建

RTFQ-PCR结果显示，与NC mimics组相比，tRF-Pro-CGG mimics组中tRF-Pro-CGG的表达水平显著升高（$P<0.001$）；与NC inhibitor组相比，tRF-Pro-CGG inhibitor组中tRF-Pro-CGG的表达水平显著降低（$P<0.001$）。Western blot结果显示，与NC mimics组相比，tRF-Pro-CGG mimics组中tRF-Pro-CGG蛋白表达水平显著升高（$P<0.01$）；与NC inhibitor组相比，tRF-Pro-CGG inhibitor组中tRF-Pro-CGG蛋白表达水平显著降低（$P<0.01$，图2）。

2.3 tRF–Pro–CGG影响小鼠胰腺癌细胞增殖

tRF-Pro-CGG mimics组中pan02细胞增殖能力与NC mimics组相比显著降低（$P<0.01$）；tRF-Pro-CGG inhibitor组中pan02细胞增殖能力与NC inhibitor组相比显著升高（$P<0.01$，图3）。

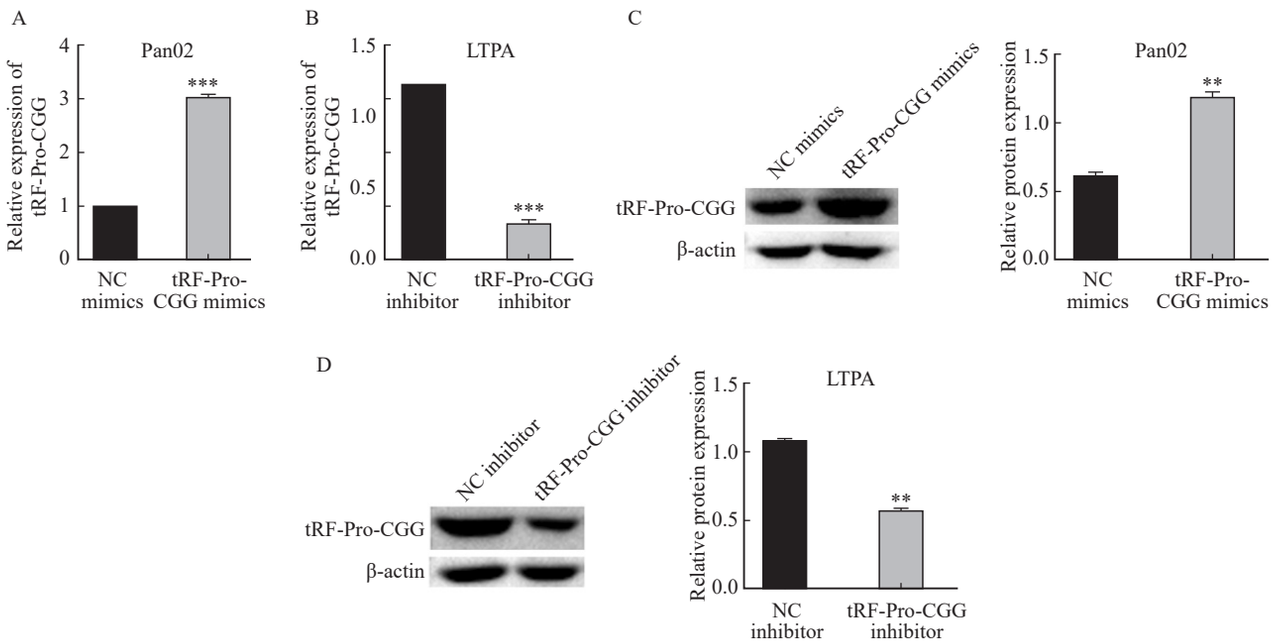

图2 过表达及敲低tRF-Pro-CGG后pan02细胞tRF-Pro-CGG表达

Fig. 2 Expression of tRF-Pro-CGG in pan02 cells after overexpression and knockdown of tRF-Pro-CGG

A, B: RTFQ-PCR was used to detect mRNA expression of tRF-Pro-CGG in each group; C, D: Western blot was used to detect protein expression of tRF-Pro-CGG in each group. ***: $P<0.001$, compared with NC mimics or NC inhibitor by RTFQ-PCR; **: $P<0.01$, compared with NC mimics or NC inhibitor by Western blot.

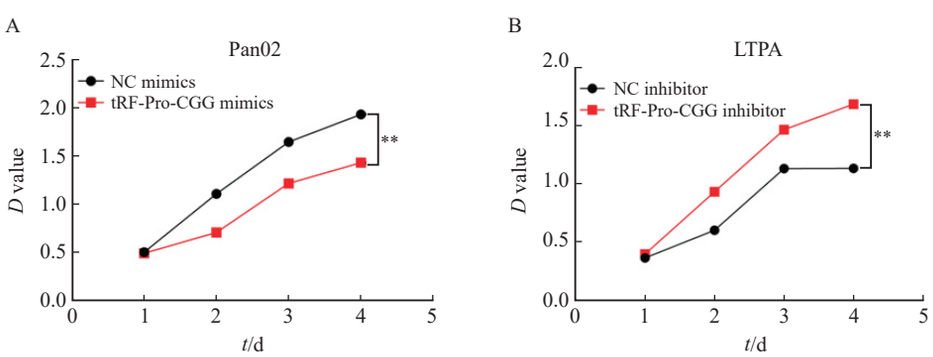

图3 过表达及敲低tRF-Pro-CGG对pan02细胞增殖的影响

Fig. 3 Effects of overexpression and knockdown of tRF-Pro-CGG on the proliferation of pan02 cells

A, B: CCK-8 was used to detect changes of cell Proliferation in each group. **: $P<0.01$, compared with NC mimics or NC inhibitor.

2.4 tRF-Pro-CGG影响小鼠胰腺癌细胞迁移和侵袭能力

与NC mimics组相比，tRF-Pro-CGG mimics组中细胞迁移和侵袭数目显著减少（$P<0.001$）；与NC inhibitor组相比，tRF-Pro-CGG inhibitor组中细胞迁移和侵袭数目显著升高（$P<0.001$，图4）。

2.5 tRF-Pro-CGG影响胰腺癌裸鼠移植瘤的生长

与NC mimics组相比，tRF-Pro-CGG mimics组中裸鼠瘤体的体积和重量均显著降低（$P<0.01$），然而两组裸鼠的体重变化差异无统计学意义（$P>0.05$）；与NC inhibitor组相比，tRF-Pro-CGG inhibitor组中裸鼠瘤体的体积和重量均显著升高（$P<0.01$），然而两组裸鼠的体重变化差异无统计学意义（$P>0.05$，图5）。

2.6 tRF-Pro-CGG对胰腺癌裸鼠移植瘤组织形态结构的影响

与NC mimics组相比，tRF-Pro-CGG mimics组坏死组织明显增多，大部分为凝固性坏死，

坏死周围可见凋亡细胞及坏死细胞残影；与NC inhibitor组相比，tRF-Pro-CGG inhibitor组坏死组织明显减少，坏死周围可见凋亡细胞及坏死细胞（图6）。

2.7　胰腺癌移植瘤组织中增殖、侵袭转移相关蛋白的表达情况

Western blot结果显示，与NC mimics组相比，tRF-Pro-CGG mimics组的移植瘤组织中Ki-67、vimentin的表达明显降低，而E-cadherin的表达明显升高（$P<0.001$）；与NC inhibitor组相比，tRF-Pro-CGG inhibitor组的移植瘤组织中Ki-67增殖指数、vimentin的表达明显升高，而E-cadherin的表达明显降低（$P<0.001$，图7）。

2.8　胰腺癌移植瘤组织中PI3K/AKT通路蛋白的相对表达量

PI3K/AKT信号通路是调节肿瘤发生、发展的重要途径之一。Western blot分析移植瘤组织中P-PI3K/AKT的表达情况显示，与NC mimics组相比，tRF-Pro-CGG mimics组的PI3K、P-PI3K、AKT和P-AKT的表达明显被抑制（$P<0.001$）；而与NC inhibitor组相比，tRF-Pro-CGG inhibitor组的PI3K、P-PI3K、AKT和P-AKT的表达明显升高（$P<0.001$，图8）。

2.9　tRF–Pro–CGG影响裸鼠胰腺癌肝转移情况

tRF-Pro-CGG mimics组4只裸鼠发生胰腺癌肝转移，1只裸鼠在4周时死亡，而NC mimics组5只裸鼠都发生胰腺癌肝转移，两组相比差异无统计学意义（$P>0.05$）；tRF-Pro-CGG inhibitor组3只发生胰腺癌肝转移，NC mimics组3只发生胰腺癌肝转移，期间1只裸鼠在3周时死亡，两组相比差异无统计学意义（$P>0.05$，图9）。

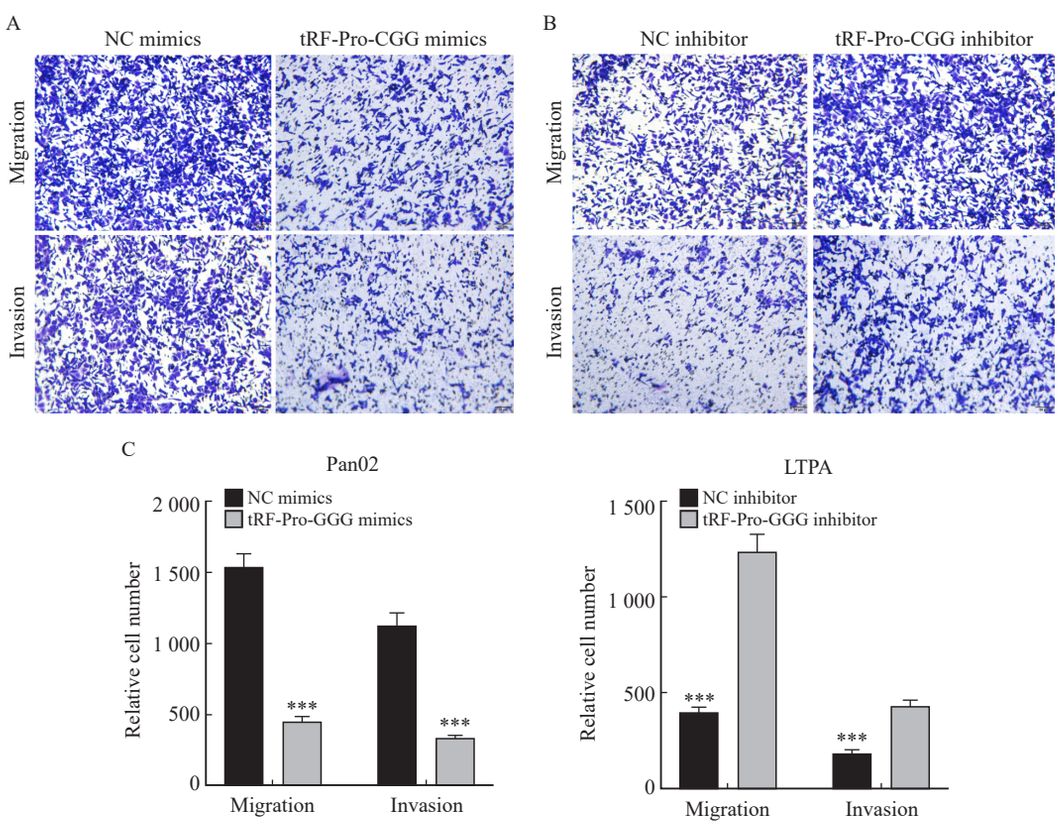

图4　过表达及敲低tRF–Pro–CGG对pan02细胞迁移和侵袭能力的影响

Fig. 4　Effects of overexpression and knockdown of tRF-Pro-CGG on migration and invasion ability of pan02 cells

A-C: Transwell was used to detect changes of cell migration and invasion in each group; ***: $P<0.001$, compared with NC mimics or NC inhibitor.

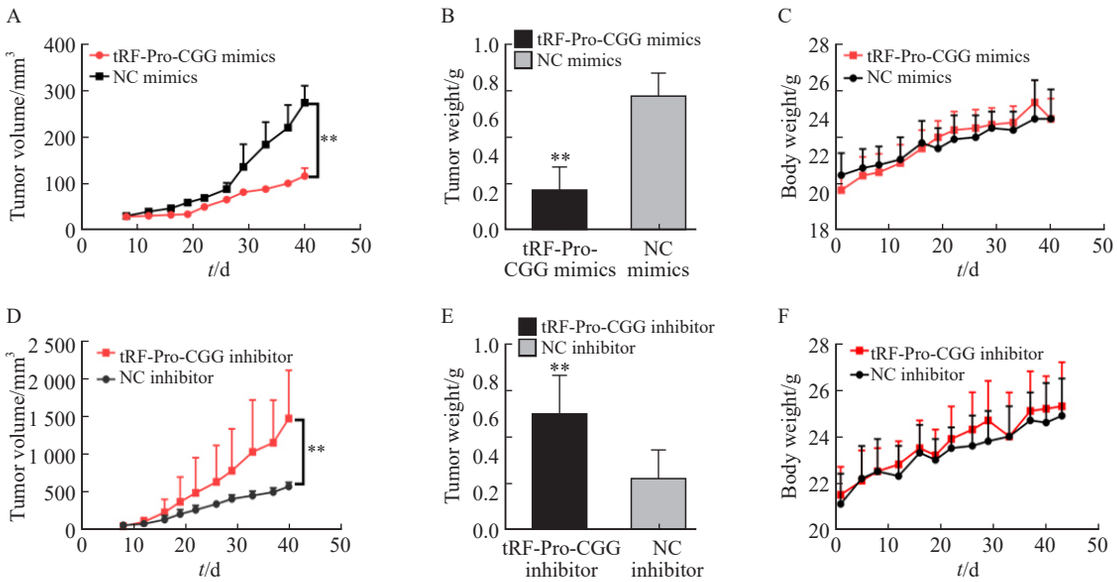

图5 过表达及敲低tRF–Pro–CGG对裸鼠胰腺癌移植瘤生长的影响

Fig. 5 Effects of overexpression and knockdown of tRF-Pro-CGG on the growth of pancreatic cancer transplant tumors in nude mice

A-C: Effects of overexpression of tRF-Pro-CGG on tumor volume, weight and body weight of nude mice; D-F: Effects of knockdown of tRF-Pro-CGG on tumor volume, weight and body weight of nude mice. **: $P<0.01$, compared with NC mimics or NC inhibitor.

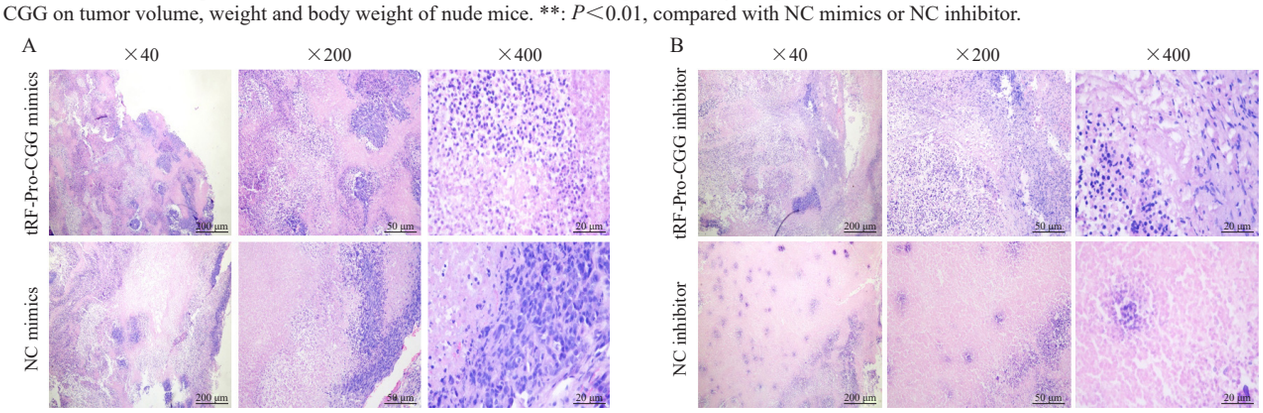

图6 过表达及敲低tRF–Pro–CGG对裸鼠胰腺癌移植瘤组织形态结构的影响

Fig. 6 Effects of overexpression and knockdown of tRF-Pro-CGG on the morphological structure of transplanted tumor tissues in nude mice with pancreatic cancer

A, B: H-E staining was used to detect changes of histomorphological structure of pancreatic cancer transplantation tumor in nude mice in each group.

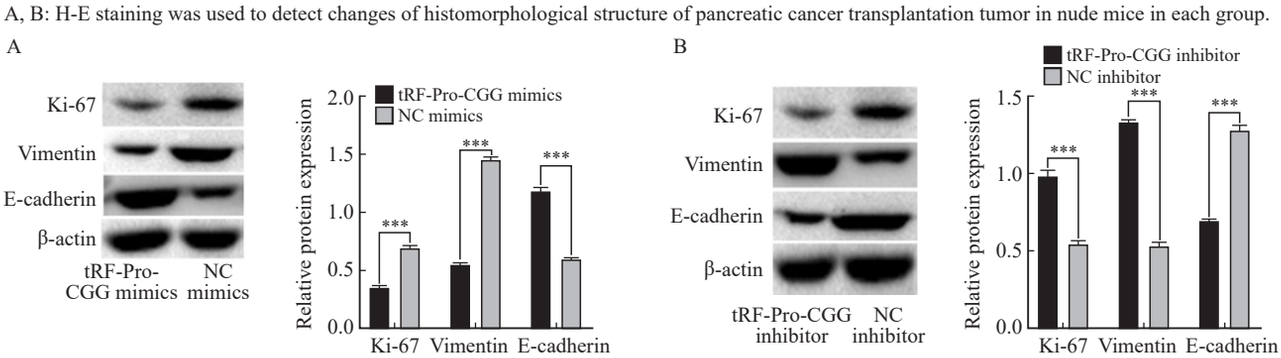

图7 过表达及敲低tRF–Pro–CGG对Ki–67增殖指数、vimentin和E–cadherin表达的影响

Fig. 7 Effects of overexpression and knockdown of tRF-Pro-CGG on Ki-67, vimentin and E-cadherin expression

A, B: Western blot was used to detect Ki-67 proliferation index, expression of vimentin and E-cadherin in each group. ***: $P<0.001$, compared with NC mimics or NC inhibitor.

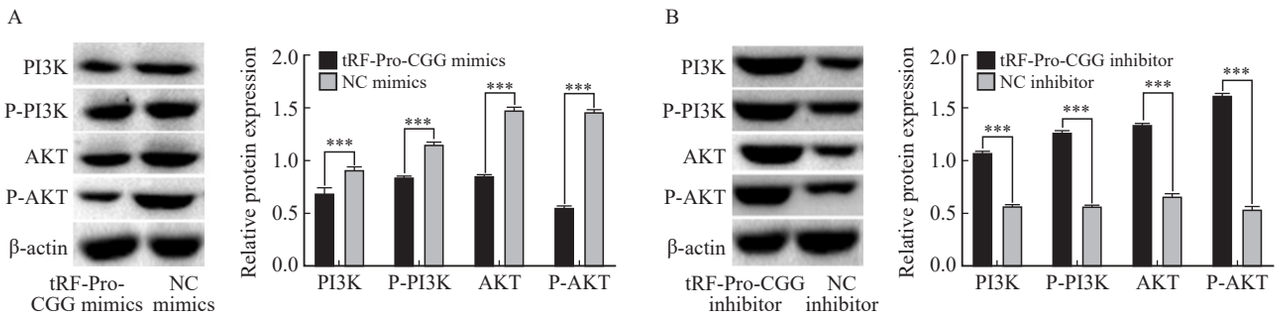

图8 过表达及敲低tRF-Pro-CGG对PI3K、P-PI3K、AKT和P-AKT表达的影响

Fig. 8 Effects of overexpression and knockdown of tRF-Pro-CGG on the expression of PI3K, P-PI3K, AKT and P-AKT

A, B: Western blot was used to detect protein expression of PI3K, P-PI3K, AKT and P-AKT in each group. ***: $P<0.01$, compared with NC mimics or NC inhibitor.

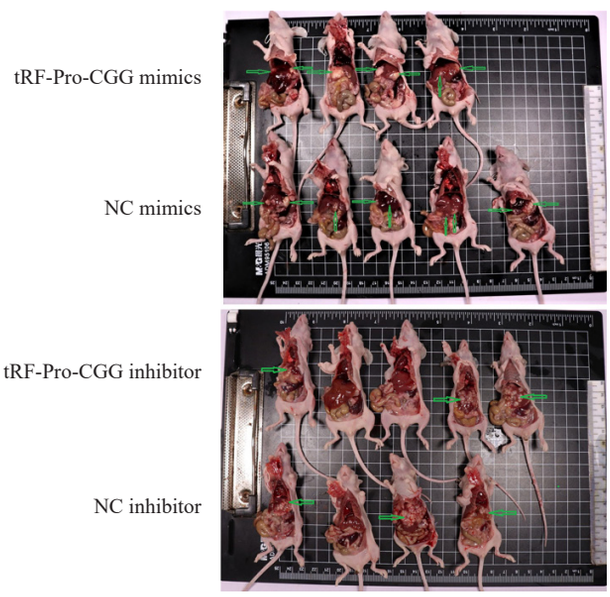

图9 过表达及敲低tRF-Pro-CGG对裸鼠胰腺癌肝转移的影响

Fig. 9 Effects of overexpression and knockdown of tRF-Pro-CGG on liver metastasis of pancreatic cancer in nude mice

3 讨 论

随着高通量二代测序技术的快速发展，大量的sncRNA尤其tRF分子被发现广泛存在于人类疾病中，如癌症、遗传性代谢疾病、神经退行性疾病及病毒感染等。tRF在不同疾病中发挥着不同的生物学功能，特别在各类肿瘤中的表达及生物发生机制也略有差异，且对于肿瘤发生、发展的探索仍停留在初步阶段。随着人们生活方式的改变及工作强度的增加，患消化道肿瘤的概率也显著提高。胰腺癌是恶性程度高、预后差的消化系统恶性肿瘤之一，其患病率和死亡率均较高，手术是治愈胰腺癌的唯一希望，但超过80%的胰腺癌患者就诊时已丧失了手术治疗的机会[6-7]。胰腺癌转移早、进展快、局部浸润等特点使其治疗效果并不显著，因此寻找新的肿瘤标志物和有效的治疗靶点非常必要。

近年来tRNA中衍生出一类独特的sncRNA，其被分为tiRNA和tRF两大类，在人体内有着重要的生物发生和肿瘤特性[8]。根据匹配的区域和长度大小，其tRF可分为tRF-1、tRF-2、tRF-3、tRF-5和i-tRF亚型[9]。tRF在许多癌症中发挥重要功能。在乳腺癌中，2-tRF可以与YBX-1相互作用，对乳腺癌细胞增殖产生抑制作用[10]。在前列腺癌中，Zeng等[11]研究发现，tRF-1001表达水平升高，促进癌细胞从G_2期到M期，提高癌细胞的增殖速度。在结直肠癌中，tRF/miR-1280可以阻止结直肠癌细胞增殖、侵袭和转移[12]。在胃癌中，tRF-38和tRF-18则充当促癌基因的角色[13]。此外，tRF-03357可明显促进卵巢癌的进程[14]。大多数tRF在缺氧环境中产生致癌应激反应，这与胰腺癌的缺氧微环境相匹配[3]，说明tRF的形成条件与胰腺癌密切相连。然而，tRF在胰腺癌中的相关机制研究报道较少。

本课题组前期研究[4]发现，tRF-Pro-CGG在人胰腺癌组织与癌旁组织中差异表达最明显，京都基因和基因组百科全书数据库（Kyoto Encyclopedia of Genes and Genomes，KEGG）功能富集分析显示，tRF-Pro-CGG下游的PI3K/AKT通路的富集密度最高，相关性最大。此外，tRF-Pro-CGG的低表达与胰腺癌患者的不良临床结局密切相关，也预示着tRF-Pro-CGG作为抑癌基因影响胰腺癌的发生、发展[5]。本研究采用tRF-

Pro-CGG转染tRF-Pro-CGG mimics或tRF-Pro-CGG inhibitor，采用RTFQ-PCR和Western blot实验检测了tRF-Pro-CGG在pan02及LTPA细胞中的表达量，CCK-8和transwell实验表明，过表达tRF-Pro-CGG可以抑制小鼠胰腺癌细胞增殖、迁移和侵袭能力。体内裸鼠移植瘤实验结果显示，过表达tRF-Pro-CGG能显著抑制瘤体重量和体积。Ki-67增殖指数可反映肿瘤细胞的增殖水平，其与癌症分期密切相关，癌症的侵袭和转移常伴随着vimentin的增加和E-cadherin的减少[15]。本研究表明，过表达tRF-Pro-CGG可有效抑制移植瘤组织中Ki-67增殖指数和vimentin的表达，而促进E-cadherin的表达。PI3K/AKT信号通路是调控肿瘤增殖和凋亡的重要通路之一，参与人类许多肿瘤的发生、发展[16]。Ebrahimi等[17]指出，PI3K/AKT信号通路在胰腺癌的发生、进展和转移中发挥着关键作用。本研究的Western blot实验证明，过表达tRF-Pro-CGG明显抑制PI3K、AKT的磷酸化水平，抑制PI3K/AKT信号通路的激活，抑制小鼠胰腺癌移植瘤的生长。

综上，过表达tRF-Pro-CGG能抑制小鼠胰腺癌细胞增殖、迁移和侵袭。过表达tRF-Pro-CGG可能通过抑制PI3K/AKT信号转导通路，从而抑制胰腺癌裸鼠移植瘤的生长。过表达tRF-Pro-CGG抑制胰腺癌移植瘤中增殖、侵袭转移蛋白的表达。本研究表明，tRF-Pro-CGG在裸鼠胰腺癌的发生、发展中发挥重要作用，有望为胰腺癌早期诊断和治疗提供新的线索。

利益冲突声明：所有作者均声明不存在利益冲突。

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