网络出版时间:2021-2-5 14:37 网络出版地址:https://kns.cnki.net/kcms/detail/34.1065.R.20210205.1049.010.html

Lnczc3h7a 对结直肠癌细胞增殖和迁移的影响及其机制

任鹏涛[1],郝英豪[1],阮红训[1],秦晓宁[1],张 苑[2],黄 炜[1]

摘要 **目的** 分析 Lnczc3h7a 在结直肠癌细胞中的表达及其对结肠癌细胞增殖和迁移的影响,并探讨其潜在的作用机制。**方法** 选择 6 种结直肠癌细胞株 SW620、SW480、HCT116、DLD-1、Caco-2、HT-29 与正常结直肠上皮细胞株 FHC,采用 RT-PCR 法检测 Lnczc3h7a 在结直肠癌细胞株及正常结直肠上皮细胞株中的表达。以 Lnczc3h7a mimics、Lnczc3h7a inhibitor 分别转染结直肠癌细胞 SW620 并分别命名为 Lnczc3h7a mimics 组、Lnczc3h7a inhibitor 组,未转染细胞作为对照组。采用 CCK8 法和细胞划痕实验分别检测 3 组细胞增殖和迁移能力差异性,采用 Western blot 法检测 Lnczc3h7a 对结肠癌细胞中 CTHRC6 蛋白表达的影响。**结果** RT-PCR 法检测结果显示,6 种结肠癌细胞株中 Lnczc3h7a 的相对表达水平均低于正常结直肠上皮细胞株 FHC,差异均有统计学意义($P<0.05$)。CCK-8 检测结果显示,转染 4、5 d 后,Lnczc3h7a mimics 组细胞增殖能力低于 Lnczc3h7a inhibitor 组和对照组($P<0.05$),Lnczc3h7a inhibitor 组细胞增殖能力高于对照组($P<0.05$)。划痕实验结果显示,在培养 24、48 h 后 3 组的伤口愈合率比较的差异均有统计学意义($F=395.524$、480.165,$P<0.05$);与对照组相比,Lnczc3h7a mimics 组细胞迁移能力降低($P<0.05$),Lnczc3h7a inhibitor 组细胞迁移能力升高($P<0.05$)。Western blot 检测结果显示,与对照组相比,Lnczc3h7a inhibitor 组中 CTHRC6 蛋白表达升高($P<0.05$),Lnczc3h7a mimics 组中 CTHRC6 蛋白表达降低($P<0.05$)。**结论** Lnczc3h7a 可能通过靶向作用于 CTHRC6 抑制结直肠癌细胞的增殖和迁移能力。

关键词 结直肠癌;Lnczc3h7a;CTHRC6;增殖;迁移
中图分类号 R 735
文献标志码 A **文章编号** 1000-1492(2021)03-0391-05
doi:10.19405/j.cnki.issn1000-1492.2021.03.011

2020-09-08 接收
基金项目:河北省卫生和健康委员会医学科学研究课题计划项目(编号:20200975)
作者单位:河北医科大学第二医院[1] 肛肠外科、[2] 心电图室 石家庄 050000
作者简介:任鹏涛,男,博士,副主任医师,责任作者,E-mail:chjf2580@163.com

结直肠癌是临床常见的恶性肿瘤,其发病率和死亡率较高,患者 5 年生存率较低[1]。结直肠癌的发生发展受多种基因及蛋白的调控[2],研究相关分子的生物学作用对解析结直肠癌的发病机制意义重大。长链非编码 RNA(long non-coding RNA,ln-

Methods Using the pFastBac DUAL plasmid as the skeleton, the pPh promoter was replaced with the CMV-SB100X-SV40 PA expression element along with the IR/DR-CMV-EGFP-SV40 PA-IR/DR sequence, and the obtained plasmid was named pBacSB-CE. Meanwhile, as a control, pBac-CE was constructed by inserting the CMV-EGFP expression element downstream of pPh promoter. The constructed recombinant plasmids were transformed into DH10Bac competent cells, and the recombinant bacmids were obtained by blue-white screening. Then the recombinant bacmids were transfected into Sf-9 insect cells by liposome, resulting in recombinant baculovirus BacSB-CE and Bac-CE. U87 cells were transduced with recombinant virus, the cell proliferation and the expression efficiency of EGFP were detected by MTT, inverted fluorescence microscopy and flow cytometry. The subcutaneous glioma tumor model of nude mice was established and the transduction efficiency of recombinant virus *in vivo* was further observed by immunofluorescence. ***Results*** The results showed that the recombinant viruses BacSB-CE and Bac-CE were successfully obtained by PCR identification. MTT results showed that the recombinant virus had no side effect on the proliferation of U87 cells. The results of inverted fluorescence microscopy and flow cytometry showed that EGFP could be expressed for at least 60 d in BacSB-CE transduced U87 cells and 10^9 a.u. total fluorescence intensity could still be detected after 15 d of transduction, while in Bac-CE transduced cells, no fluorescence cells and fluorescence signal could be detected after 15 d. ***Conclusion*** In this study, the SB transposon-mediated baculovirus gene delivery system was successfully constructed. The recombinant virus has a good biosafety and can be sustained and stable expressed *in vitro* and *in vivo* of mammals.

Key words sleeping beauty transposon; baculovirus; U87 cells; sustained expression

cRNA)可对多种疾病的生理、病理过程产生影响[3]。Lnczc3h7a是近年来新发现的lncRNA家族成员,其参与某些肿瘤的发生和发展[4],但作用机制目前还鲜有报道。胶原三股螺旋重复蛋白6(collagen triple helix repeat protein 6,CTHRC6)是一种分泌性糖蛋白,具有促进细胞迁移和减少胶原沉积的功能[5]。研究[6-7]显示CTHRC6在结直肠癌、食管癌等实体瘤中异常表达,可能与肿瘤的侵袭、迁移有关。该研究拟分析Lnczc3h7a在结直肠癌细胞中的表达及其对结直肠癌细胞增殖和迁移的影响,并探讨其作用机制。

1 材料与方法

1.1 材料 6种结直肠癌细胞株(SW620、SW480、HCT116、DLD-1、Caco-2、HT-29)及正常结直肠上皮细胞系(FHC)均购自北京中国医学科学院基础医学研究所;鼠抗人CTHRC6抗体及GAPDH抗体购自美国Santa Cruz公司;Lnczc3h7a模拟物(Lnczc3h7a mimics)和Lnczc3h7a抑制物(Lnczc3h7a inhibitor)购自广州锐博生物有限公司;脂质体Lipofectamine 2000购自美国Invitrogen公司;RNA提取试剂盒、RT-PCR反转录试剂盒及荧光定量试剂盒购自日本Takara公司;荧光定量PCR仪7500购自美国ABI公司;酶标仪PerkinElmer购自美国Enspire公司。

1.2 方法

1.2.1 RT-PCR法检测Lnczc3h7a在结直肠癌细胞中的表达 按照RNA提取试剂盒说明书的操作步骤,提取6种结直肠癌细胞株和正常结直肠上皮细胞中的总RNA。按照RT-PCR反转录试剂盒说明书合成cDNA。按照荧光定量试剂盒说明书,使用ABI 7500 PCR仪进行RT-PCR反应。采用$2^{-\triangle\triangle CT}$方法计算Lnczc3h7a的表达水平,以GAPDH为内参。

1.2.2 细胞转染 取对数生长期的SW620细胞分别接种于3个T75培养瓶。待细胞融合率达70%~80%时,按照Lipofectamine 2000转染试剂盒说明书将Lnczc3h7a mimics和Lnczc3h7a inhibitor转染细胞分别作为Lnczc3h7a mimics组和Lnczc3h7a inhibitor组,将未转染细胞作为对照组。转染48 h后,收集细胞进行后续实验。

1.2.3 CCK-8法检测Lnczc3h7a对结直肠癌细胞增殖的影响 将如上Lnczc3h7a mimics组、Lnczc3h7a inhibitor组、对照组收集的细胞以5×10^6/L密度接种于96孔板中,待细胞贴壁后,加入CCK-8溶液和含10%新生牛血清的1640完全培养基,于37℃、5% CO_2饱和湿度的培养箱中培养。分别于1、2、3、4、5 d时间点检测细胞的增殖能力,并绘制细胞生长曲线。

1.2.4 划痕实验检测Lnczc3h7a对结直肠癌细胞迁移能力的影响 将如上Lnczc3h7a mimics组、Lnczc3h7a inhibitor组、对照组收集的细胞以2×10^8个/L密度接种于6孔板中,置于CO_2培养箱进行培养。待细胞融合率达到90%以上,使用无菌枪头在细胞培养皿中进行划痕,经无菌PBS洗涤后,加入含0.5%新生牛血清的1640完全培养基,于37 ℃、5% CO_2饱和湿度的培养箱中培养,分别采集0、24、48 h时间点的细胞迁移图像,并计算迁移率。

1.2.5 Western blot检测CTHRC6蛋白的表达 收集3组细胞,经裂解后提取细胞总蛋白,BCA法测定蛋白浓度,取各组蛋白样品进行SDS-PAGE电泳,湿转至硝酸纤维素膜上,使用脱脂奶粉室温封闭2 h,分别加入按照CTHRC6抗体(1∶1 000),4 ℃摇床孵育过夜;次日,经TBS-T洗涤3次后,加入HRP标记的山羊抗小鼠IgG(1∶5 000),室温摇床孵育2 h,经TBS-T洗涤3次后,使用化学发光底物试剂检测目的蛋白,曝光X线胶片,并利用Quantity One软件进行图像分析。

1.3 统计学处理 采用SPSS20.00分析实验数据,所有实验数据均为重复实验3次后获得的数据。进行方差齐性检验、正态性检验。计量资料实验数据以$\bar{x}\pm s$表示。单变量两组资料之间的比较采用t检验;多组资料之间的比较采用单因素方差分析,以$P<0.05$为差异有统计学意义。

2 结果

2.1 Lnczc3h7a在结直肠癌细胞株中的表达情况 RT-PCR法检测结果显示6种结肠癌细胞株SW620、SW480、HCT116、DLD-1、Caco-2、HT-29中Lnczc3h7a的相对表达水平均低于正常结直肠上皮细胞FHC,差异均有统计学意义($P<0.05$),见图1。3组的Lnczc3h7a的相对表达水平比较见图2。

2.2 Lnczc3h7a对结直肠癌细胞增殖能力的影响 CCK-8检测结果显示:转染1~3 d后,3组之间细胞增殖能力差异无统计学意义($P>0.05$);转染4~5 d后,Lnczc3h7a mimics组细胞增殖能力低于Lnczc3h7a inhibitor组和对照组($P<0.05$),Lnczc3h7a inhibitor组细胞增殖能力高于对照组($P<0.05$),见图3。

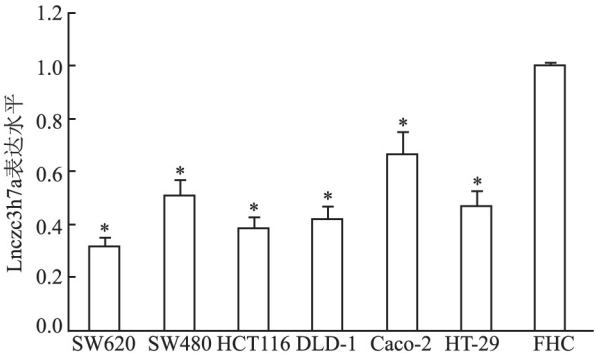

图1 Lnczc3h7a 在结直肠癌细胞株中的表达情况

与 FHC 细胞比较: * $P<0.05$

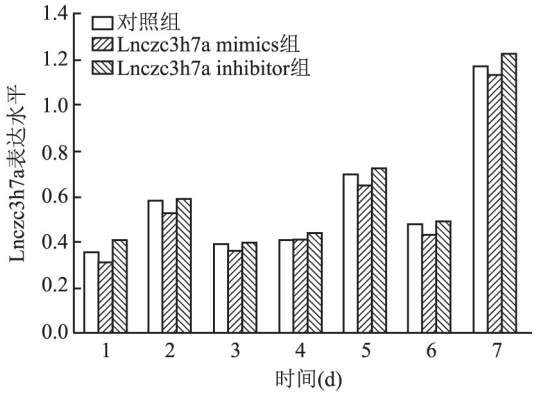

图2 3 组的 Lnczc3h7a 表达水平比较

2.3 Lnczc3h7a 对结直肠癌细胞迁移能力的影响 划痕实验结果显示,在培养 24、48 h 后,对照组的伤口愈合率分别为(72.10±6.12)%、(59.73±8.32)%,Lnczc3h7a mimics 组的伤口愈合率分别为(82.29±5.03)%、(72.81±6.27)%,Lnczc3h7a inhibitor 组的伤口愈合率分别为(59.42±5.73)%、(38.69±7.64)%,3 组比较的差异均有统计学意义($F=395.524$、480.165,$P<0.05$);与对照组相比,Lnczc3h7a mimics 组细胞迁移能力降低($P<0.05$),Lnczc3h7a inhibitor 组细胞迁移能力升高($P<0.05$),见图 4。

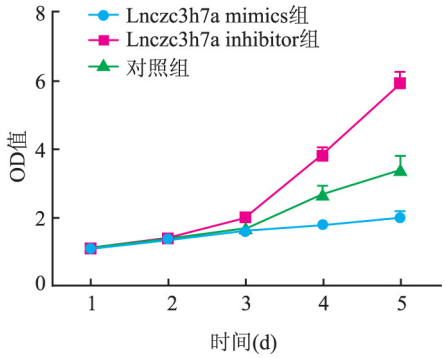

图3 Lnczc3h7a 对结直肠癌细胞增殖能力的影响

2.4 Lnczc3h7a 对结直肠癌细胞中 CTHRC6 蛋白表达的影响 生物信息学分析显示,Lnczc3h7a 与 CTHRC6 蛋白具有相似的结合位点,二者可能存在内在调控关联,为了进一步验证 Lnczc3h7a 对 CTHRC6 蛋白表达的影响,该研究检测了 Lnczc3h7a mimics 组、Lnczc3h7a inhibitor 组、对照组中 CTHRC6 蛋白的表达情况,Western blot 检测结果显示,与对照组相比,Lnczc3h7a inhibitor 组中 CTHRC6 蛋白表达升高($P<0.05$),Lnczc3h7a mimics 组中降低($P<0.05$),见表 1、图 5。

3 讨论

结直肠癌是消化系统最常见的恶性肿瘤,近年

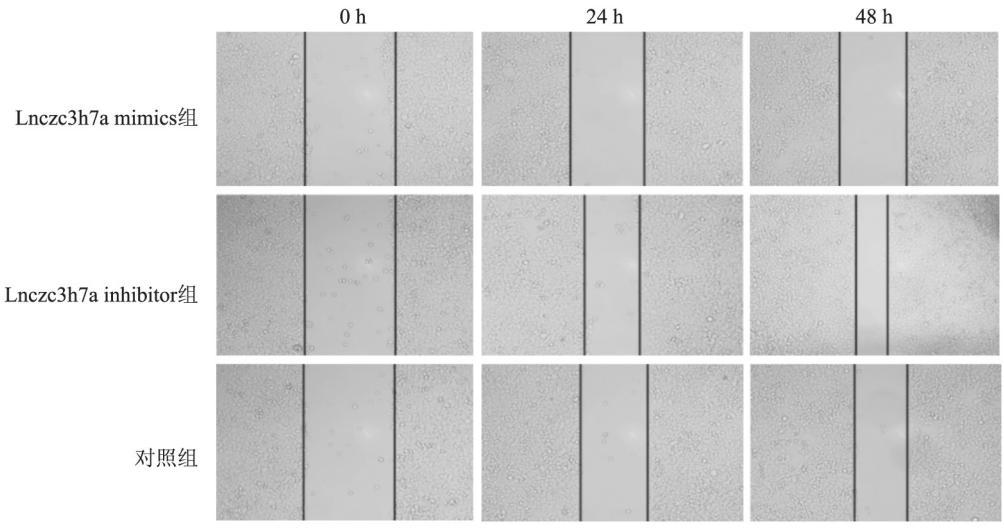

图4 划痕实验法检测 Lnczc3h7a 对结肠癌细胞迁移能力的影响

表 1 3 组各肿瘤细胞株中的 CTHRC6 蛋白表达水平($n=3$, $\bar{x}\pm s$)

组别	SW620	SW480	HCT116	DLD-1	Caco-2	HT-29
对照	31.28±5.92	34.89±6.22	29.60±6.37	30.51±5.18	35.68±6.26	32.75±5.49
Lnczc3h7a mimics	12.13±3.14*	12.25±3.09*	12.20±3.31*	12.43±3.26*	12.55±3.62*	12.76±3.25*
Lnczc3h7a inhibitor	69.05±5.27*	68.36±5.16*	68.79±5.05*	69.23±5.49*	69.43±5.27*	69.16±5.30*
F 值	2382.64	2346.41	2381.93	2306.03	2312.73	2324.18
P 值	0	0	0	0	0	0

与对照组比较: * $P<0.05$

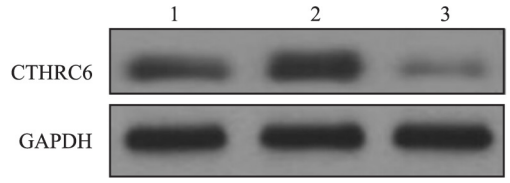

图 5 Western blot 法检测 Lnczc3h7a 对结直肠癌细胞中 CTHRC6 蛋白表达的影响

1: 对照组; 2: Lnczc3h7a inhibitor 组; 3: Lnczc3h7a mimics 组

我国结直肠癌的发病率和病死率均呈增高趋势[8]。目前所知,结直肠癌的发生发展是一个多因素参与的复杂生物学过程。探究结直肠癌发生发展过程中所涉及的关键分子的生物学作用,对于结直肠癌的临床治疗具有重要意义。

lncRNA 是一类长约 220 个核苷酸的非编码长链 RNA,其可在表观遗传学、基因组转录水平及转录后水平等多个层面上调控基因的表达[9-10]。Lnczc3h7a 是近年发现的一种新的 lncRNA,其在头颈部肿瘤、结直肠癌、胃癌、神经母细胞瘤、肾癌等多种肿瘤细胞中的表达存在很大的差异性[11-12]。最近有研究[13]表明,Lnczc3h7a 在某些高侵袭能力的肿瘤细胞系中表达下调。另有研究[14]显示 Lnczc3h7a 与结直肠癌患者病情的恶性程度有关,但其在结直肠癌中的作用尚不清楚。本研究首先分析了 Lnczc3h7a 在结肠直癌细胞株和正常结直肠上皮细胞中表达的差异性,结果显示 6 种结肠癌细胞株 SW620、SW480、HCT116、DLD-1、Caco-2、HT-29 中 Lnczc3h7a 的相对表达水平均低于正常结直肠上皮细胞株 FHC($P<0.05$),这一结果表明 Lnczc3h7a 可能参与了结直肠癌的发生发展过程。由于 Lnczc3h7a 在 SW620 细胞中表达最低,因此本研究中选取 SW620 细胞株作为后续实验细胞株。为了进一步研究 Lnczc3h7a 在结直肠癌中的作用,本研究通过向 SW620 细胞分别转染 Lnczc3h7a mimics 和 Lnczc3h7a inhibitor,实现调控 Lnczc3h7a 的表达水平,结果显示,与对照组相比,Lnczc3h7a mimics 组细胞增殖能力和迁移能力降低($P<0.05$),Lnczc3h7a inhibitor 组细胞增殖能力和迁移能力升高($P<0.05$),表明 Lnczc3h7a 可能是通过抑制结直肠癌细胞的增殖和迁移而发挥抑癌基因的作用。

该研究通过生物信息学软件预测了 Lnczc3h7a 的靶基因,结果显示 Lnczc3h7a 与 CTHRC6 具有相似的结合位点,二者可能存在内在调控关联。CTHRC6 蛋白是一种分泌性糖蛋白,具有促进细胞迁移和减少胶原沉积的功能。最近有研究[15]表明,CTHRC6 在结直肠癌细胞中高表达,靶向沉默 CTHRC6 表达可降低结直肠癌细胞的增殖能力。为了验证 Lnczc3h7a 对结直肠癌细胞中 CTHRC6 蛋白表达的影响,该研究检测了 Lnczc3h7a mimics 组、Lnczc3h7a inhibitor 组、对照组中 CTHRC6 蛋白的表达情况,结果显示,与对照组相比,Lnczc3h7a inhibitor 组中 CTHRC6 蛋白表达升高($P<0.05$),Lnczc3h7a mimics 组中 CTHRC6 蛋白表达降低($P<0.05$),这一结果表明,Lnczc3h7a 对结直肠癌 CTHRC6 蛋白表达具有抑制作用。因此推测 Lnczc3h7a 可能是通过靶向作用于 CTHRC6 抑制结直肠癌细胞的增殖和迁移能力。

Lnczc3h7a 在结肠直肠癌细胞株中表达下调,Lnczc3h7a 过表达可抑制结直肠癌细胞的增殖和迁移能力,Lnczc3h7a 可能通过靶向作用于 CTHRC6 而抑制结直肠癌细胞增殖和迁移。

参考文献

[1] 于永扬,陈海宁,周总光. 我国结直肠癌的现状、制约瓶颈与反思[J]. 中国普外基础与临床杂志,2019,12(8):32-5.

[2] Zhang Y, Sui R, Chen Y, et al. Long noncoding RNA MT1JP inhibits proliferation, invasion, and migration while promoting apoptosis of glioma cells through the activation of PTEN/Akt signaling pathway[J]. J Cellul Phys, 2019, 234(11):19553-64.

[3] Zhou M, Sun Y, Sun Y, et al. Comprehensive analysis of lncRNA expression profiles reveals a novel lncRNA signature to discriminate nonequivalent outcomes in patients with ovarian cancer[J]. Oncot, 2016, 7(22):32433-48.

[4] Oshiumi H. Recent advances and contradictions in the study of the Iindividual roles of ubiquitin ligases that regulate RIG-I-like receptor-mediated antiviral innate immune responses[J]. Front Immunol, 2020, 11:1296.

[5] Tan F, Liu F, Liu H, et al. CTHRC6 is associated with peritoneal carcinomatosis in colorectal cancer: a new predictor for prognosis [J]. Med Oncol, 2013, 30(1): 473.

[6] Jiang K, Dong C, Yin Z, et al. The critical role of exosomes in tumor biology [J]. J Cell Biochem, 2018.

[7] Li N, Ding H, Li Z, et al. Effect of high-fat diet-induced obesity on the small-conductance Ca^{2+}-activated K^+ channel function affecting the contractility of rat detrusor smooth muscle [J]. Int Urol Nephrol, 2019, 51(1): 61 - 72.

[8] 郭天安，谢 丽，赵 江，等. 中国结直肠癌1988 - 2009年发病率和死亡率趋势分析 [J]. 中华胃肠外科杂志, 2018, 21(1): 33 - 40.

[9] Engreitz J M, Haines J E, Perez E M, et al. Local regulation of gene expression by lncRNA promoters, transcription and splicing [J]. Nature, 2016, 539(7629): 452 - 5.

[10] Zou Z W, Ma C, Medoro L, et al. LncRNA ANRIL is up-regulated in nasopharyngeal carcinoma and promotes the cancer progression via increasing proliferation, reprograming cell glucose metabolism and inducing side-population stem-like cancer cells [J]. Oncotarget, 2016, 7(38): 61741 - 54.

[11] Qin L, Min W, Xin S. AIP1 suppresses transplant arteriosclerosis through inhibition of vascular smooth muscle cell inflammatory response to IFNγ [J]. AnatRec (Hoboken), 2019, 302(9): 1587 - 93.

[12] Yang J, Xu J, E Y, et al. Predictive and prognostic value of circulating blood lymphocyte subsets in metastatic breast cancer [J]. Cancer Med, 2019, 8(2): 492 - 500.

[13] Lin H, Jiang M, Liu L, et al. The longnoncoding RNA Lnczc3h7a promotes a TRIM25-mediated RIG-I antiviral innate immunereresponse [J]. Nat Immunol, 2019, 20(7): 812 - 23.

[14] Wang X, He J, Chen X, et al. Stenting as a bridge to resection versus emergency surgery for left-sided colorectal cancer with malignant obstruction: a meta-analysis [J]. Int J Surg, 2017, 48: 64 - 8.

[15] Li J, Cao Y F, Sun X Y, et al. Plasma tyrosine and its interaction with low high-density lipoprotein cholesterol for type 2 diabetes mellitus in Chinese [J]. J DiabetesInvestig, 2019, 10(2): 491 - 8.

Effect of lnczc3h7a on proliferation and migration of colorectal cancer cells and its mechanism

Ren Pengtao, Hao Yinghao, Ruan Hongxun, et al

(*Anorectal Surgery, The Second Hospital of Hebei Medical University, Shijiazhuang* 050000)

Abstract ***Objective*** To analyze the expression of Lnczc3h7a in colorectal cancer cells and its effect on the proliferation and migration of colorectal cancer cells, and explore its potential mechanism. ***Methods*** Six colorectal cancer cell lines including SW620、SW480、HCT116、DLD-1、Caco-2、HT-29 and normal colorectal epithelial cell FHC were selected. The expression of Lnczc3h7a in colorectal cancer cell lines and normal colorectal epithelial cells was detected by RT-PCR. Lnczc3h7a mimics and Lnczc3h7a inhibitor were used to transfect colorectal cancer cells SW620 respectively and named as Lnczc3h7a mimics group and Lnczc3h7a inhibitor group respectively, and the untransfected cells were used as the control group. CCK-8 method and cell scratch test were used to detect the difference of cell proliferation and migration ability among the three groups, and Western blot method was used to detect the effect of Lnczc3h7a on the expression of CTHRC6 protein in colorectal cancer cells. ***Results*** The results of RT-PCR showed that the relative expression levels of Lnczc3h7a in the six colon cancer cell lines were significantly lower than those of normal colorectal epithelial cells FHC, and all were statistically significant ($P<0.05$). CCK-8 test results showed that the cell proliferation ability of the Lnczc3h7a mimics group was significantly lower than that of the Lnczc3h7a inhibitor group and the control group ($P<0.05$), and the cell proliferation ability of the Lnczc3h7a inhibitor group was significantly higher than that of the control group after 4 and 5 days of transfection. The scratch test results showed that the difference of wound healing rate among the three groups was statistically significant ($F=395.524, 480.165$, $P<0.05$); compared with the control group, the cell migration ability of the Lnczc3h7a mimics group significantly reduced ($P<0.05$), and the cell migration ability of the Lnczc3h7a inhibitor group significantly increased ($P<0.05$). Western blot results showed that compared with the control group, the expression of CTHRC6 protein in the Lnczc3h7a inhibitor group significantly increased ($P<0.05$), and the expression of CTHRC6 protein in the Lnczc3h7a mimics group significantly reduced ($P<0.05$). ***Conclusion*** Lnczc3h7a may inhibit the proliferation and migration of colorectal cancer cells by targeting CTHRC6.

Key words colorectal cancer; Lnczc3h7a; CTHRC6; proliferation; migration