

miR-31 通过激活 NF- κ B 信号通路而促进结肠癌细胞增殖

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摘要 微小 RNA (microRNA) 在肿瘤的发生发展中发挥重要作用。有研究表明, 在结肠癌患者肿瘤组织中, miR-31 表达水平增高。然而, 定量 PCR 只能检测所在组织 miR-31 的整体表达水平, 而无法观察 miR-31 在特定组织与特定细胞中的表达分布。目前, 尚未见关于 miR-31 在结肠癌中原位表达的报道。本文从研究 miR-31 在结肠癌中的原位表达入手, 进一步探究 miR-31 在结肠癌细胞中的功能及作用机制。原位杂交实验结果显示, miR-31 在结肠癌肿瘤细胞中的原位表达明显升高; 体外过表达或敲减 miR-31 证实, 其可以促进结肠癌细胞增殖和集落形成; 荧光定量 PCR 与 Western 印迹和双荧光素酶报告基因实验证实, 在结肠癌细胞中, NF- κ B 通路的抑制因子丝氨酸/苏氨酸激酶 40 (STK40) 是 miR-31 下游靶基因, miR-31 靶向作用于 STK40 而激活 NF- κ B 通路; 反之, 抑制 NF- κ B 通路, miR-31 的促增殖能力明显下降。上述结果提示, miR-31 可能通过激活 NF- κ B 信号通路而促进结肠癌细胞的增殖。

关键词 miR-31; 结肠癌细胞; NF- κ B 信号通路; 丝氨酸/苏氨酸激酶 40

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miR-31 Promotes the Proliferation of Colorectal Cancer Cells Through Activating NF- κ B Signal Pathway

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Abstract microRNAs plays an important role in the pathogenesis of cancers. It has been shown by qRT-PCR testing that miR-31 is highly expressed in colorectal cancers of patients. However, qRT-PCR can detect the expression only in the whole cancer samples but not in the local expression of miR-31 in cancer tissues. So far, the *in situ* expression of miR-31 in colorectal cancer has not been identified yet. The aim of this study was to investigate the *in situ* expression of miR-31 in colorectal cancer, and to further explore the role of miR-31 *in vitro*. The results showed that *in situ* expression of miR-31 in colorectal cancer cells was significantly increased by *in situ* hybridization method. miR-31 expression was detected using *in situ* hybridization in colorectal cancer tissues. The role of miR-31 was analyzed in colorectal cancer cells using cell proliferation assay and colony formation assay. The underlying mechanisms were further explored. The results showed that *in situ* expression of miR-31 in colorectal cancer cells was

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significantly increased, and the majority of miR-31 was located in colorectal cancer cells. Functional assays confirmed that miR-31 could promote the proliferation and clonogenic ability of colorectal cancer cells by transfecting the mimics and/or inhibitors of miR-31 *in vitro*. Serine/threonine kinase 40 (STK40) was identified as the target of miR-31 in colorectal cancer cells by qRT-PCR, Western blotting and luciferase assay. miR-31 could activate NF- κ B signaling pathway by targeting STK40. Upon inhibition of NF- κ B signal pathway, the proliferative ability of miR-31 was dramatically decreased. In conclusion, the activation of NF- κ B signaling pathway might be partially account for the mechanisms of miR-31 in promoting proliferation in colorectal cancer cells.

Key words miR-31; colorectal cancer; NF- κ B signal pathway; serine/threonine kinase 40

结肠癌是人类主要恶性肿瘤之一。在美国,其结肠癌发病率和死亡率位于第3位,在中国,结肠癌发病率和死亡率均位于第5位^[1-2]。近年来,人们发现的微小RNA(microRNAs)参与结肠癌的发生与发展^[3],并在肿瘤的诊断、治疗效果乃至预后评价中具有潜在的价值^[4]。microRNAs通过靶向作用于抑癌基因(如p53)从而发挥抑癌基因的作用,或靶向作用于癌基因(如myc)发挥抑癌基因的作用^[5-6]。

microRNA-31(miR-31)基因位于人类9p21.3的染色体条带上,该位点是多种肿瘤缺失的热点片段。已经显示,miR-31基因座的缺失发生在黑色素瘤、间皮瘤、不同类型的白血病以及尿路上皮癌等多种肿瘤中,而且在胚胎着床、发育、免疫调节和肿瘤发生发展中也发挥重要功能。miR-31已经被证实参与诸多肿瘤的发病进展,包括结肠癌^[7]、胃癌^[8]、宫颈癌^[9]等。本研究从人结肠癌病理标本的荧光原位杂交入手,检测miR-31在特定病理组织与细胞中的表达情况,并进一步探索其在体外对结肠癌细胞的增殖的影响及可能的机制。

本文旨在进一步探究miR-31在结肠癌组织中的具体表达部位,并通过体外实验探究miR-31对结肠癌细胞的调节作用及可能的机制。

1 材料与方法

1.1 患者样本

所有结肠癌组织来源于北京大学医学部病理学系,所有的病人组织在手术后立刻固定包埋,并保存在-80℃。结肠癌均由北京大学医学部病理学系确诊,病理类型为溃疡型中分化结肠癌。所有参与者均知情同意,研究得到符合负责人体试验委员会所制定的伦理学标准并得到该委员会的批准。

1.2 原位杂交

所用试剂及容器均使用DEPC水或DEPC水处理。

应用结肠癌石蜡切片(10 μm厚)经过二甲苯及酒精脱蜡后,使用10 μL/mL蛋白酶K(Amresco) 0.2%甘氨酸4%多聚甲醛去蛋白质,在杂交溶液中60℃预杂交2h,加入DIG标记的miRCURY LNA探针(EXIQON)60℃杂交16h。洗涤后4℃孵育抗DIG-AP Fab片段(Roche)过夜,加入NBT/BCIP反应48h,洗涤脱水后封片并拍照。

1.3 细胞培养

HCT116和LoVo细胞来源于中国医学科学院基础医学研究所基础医学细胞中心,HCT116和LoVo细胞使用10%胎牛血清的DMEM培养基,所有的细胞均在5%CO₂孵箱中37℃培养。

1.4 RNA提取及荧光定量PCR实验

TRIzol(Invitrogen)提取全细胞RNA,反转录试剂盒及SYBR染料购自天根生化科技有限公司,miR-31的引物序列为上游5'-GCCGAGGCAAGATGCTGGCA-3',下游为通用引物,U6的引物序列为上游5'-CTCGCTTCGGCAGCACA-3',下游5'-AACGCTTCACGAATTTGCGT-3',STK40的引物序列为上游5'-CAGCACTACGTCATCAAGGAG-3',下游5'-CGATGTGTCTCTTGTGAGC-3',GAPDH的引物序列为上游5'-GGAGCGAGATCCCTCCAAAAT-3',下游5'-GGCTGTTGTCATACTTCTCATGG-3',引物序列均来源于primerbank(<https://pga.mgh.harvard.edu/primerbank/>),由上海生物工程有限公司合成。

1.5 细胞增殖实验

将细胞接种在96孔板中,每孔细胞3000个,每组6个副孔,分为5组。从第2d细胞贴壁开始,连续5d,每天换液,并在每孔中加入20 μL MTS试剂(Promega)37℃继续培养3h。于酶标仪测吸光值,波长设置为490 nm,并将5d数值绘制增殖曲线。细胞数量与形成的四唑化合物[3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; MTS]的吸光值成正比。

1.6 集落形成实验

将细胞接种于 6 孔板中,每孔种 9 000 个细胞,置于 37 ℃ 培养 10 ~ 14 d,按照 3:1 配置的乙醇:冰乙酸固定液,室温固定 20 min,弃固定液,用 0.1% 亚甲蓝溶液室温染色 20 min,室温晾干后拍照并计数集落形成数量。

1.7 miR-31 mimics 及 inhibitor

miR-31 mimics 及 inhibitor 及对应的阴性对照,均购自广州市锐博生物科技有限公司。6 孔板转染时的细胞密度为 70% ~ 80%,转染试剂为 lipofectamine 2000 (Invitrogen)。

1.8 细胞免疫荧光检测

将细胞接种到预先放置盖玻片的 6 孔板培养皿中,贴壁后用 PBS 洗 2 次(每次 5 min);多聚甲醛室温固定 2 min,PBS 洗涤 3 次;0.5% Triton X-100 室温通透 20 min,通透后 PBS 洗涤 3 次;加入正常山羊血清 200 μ L,室温封闭 30 min;加入一抗 4 ℃ 孵育过夜;次日取出后 PBS 洗涤 3 次后,加入荧光二抗,37 ℃ 孵育 1 h,PBS 浸洗切片 3 次;滴加 DAPI 避光孵育 5 min,PBS 浸洗切片 3 次;用封片剂封片,然后在荧光显微镜下观察采集图像。

1.9 蛋白质提取及 Western 印迹

转染 48 h 后,RIPA 液裂解细胞,将待测蛋白质进行定量。蛋白免疫印迹杂交实验电压 80 V 1 h,电压 120 V 1 h;200 mA 恒流,冰浴电转移 2 h;NC 膜置于 5% BSA 室温孵育 1 h;一抗 4 ℃ 孵育过夜;次日,用 TBST 漂洗 3 次(每次 10 min),置于含荧光二抗(IRdye700,IRDye800)的封闭缓冲液室温避光孵育 45 min,TBST 漂洗 3 次(每次 10 min),Odyssey 红外激光成像系统(LI-COR Infrared Imaging Systems)扫描 NC 膜。抗 p65 的一抗浓度为 1:500 (Santa Cruz Biotechnology),抗 STK40 的一抗浓度为 1:1 000 (Abcam),抗 GAPDH 一抗浓度为 1:2 000 (Cell Signaling Technology),抗兔或抗小鼠二抗浓度为 1:5 000 (Odyssey)。

1.10 双荧光素酶报告基因检测

通过 PCR 扩增包含预测与 miR-31 结合的 STK40 3'-UTR 片段,克隆到 pmirGLO 双荧光素酶报告质粒。使用点突变试剂盒,突变预测的 STK40 3'-UTR 靶点。将 pmirGLO-STK40 3'-UTR 质粒或突变质粒与 miR-31 mimics 共转染到 293T 细胞中,48 h 后检测荧光素酶活性。萤火虫荧光素酶(luc2)活性为监测 miRNA 调节作用的主要酶活性,海肾萤光素

酶(hRluc-neo)作为对照。

1.11 统计学方法

所有的实验均重复至少 3 次。使用配对样本 *t* 检验方法分析不同组别之间的差异,*P* 值小于 0.05 视为统计学有差异,所有的数据均使用 SPSS 21.0 软件分析。

2 结果

2.1 miR-31 在结肠癌组织中原位高表达

肿瘤组织病理类型均为中分化结肠腺癌,HE 染色清楚区分癌组织和癌旁组织:癌组织中癌细胞核大,核深染,癌组织呈现不规则的腺腔样排列,而癌旁组织相对核淡染(Fig. 1A, B, C)。原位杂交实验采用 miR-31 探针特异性的检测结肠癌组织中 miR-31 的表达,同时 scrambled 探针(Fig. 1D)和 U6 探针(Fig. 1F)分别作为阴性对照和阳性对照。结果显示,在直肠溃疡型中分化结肠癌中,miR-31 在结肠癌肿瘤细胞中的原位表达明显升高,结肠癌组织中的 miR-31 基本都表达于肿瘤细胞内(Fig. 1E)。

2.2 miR-31 促进结肠癌细胞增殖

为了进一步研究原位表达增高的 miR-31 在结肠癌中的作用,两种结肠癌细胞 HCT116 和 LoVo 被用来进行体外功能研究。将 miR-31 mimics 和 inhibitor 分别转染结肠癌细胞,荧光定量 PCR(qRT-PCR)实验证实,miR-31 mimics 和 inhibitor 可以分别提高和减弱 HCT116 和 LoVo 细胞中 miR-31 的表达水平。结果显示:在 LoVo 细胞中,与对照组相比,转染 miR-31 mimics 后 miR-31 的表达增高 5.02 ± 0.17 倍;转染 miR-31 inhibitor 后 miR-31 表达下降为 $14\% \pm 2\%$ (Fig. 2A);在 HCT116 细胞中,转染 miR-31 mimics 后,miR-31 的表达增高 8.24 ± 0.57 倍;转染 miR-31 inhibitor 后,miR-31 的表达下降为对照组的 $13\% \pm 1\%$ (Fig. 2B)。在 LoVo 细胞中,细胞生长曲线显示,过表达 miR-31 明显促进结肠癌细胞增殖(Fig. 2C),敲减 miR-31 抑制结肠癌增殖(Fig. 2D);在 HCT116 细胞中也观察到同样的现象(Fig. 2E, F)。

2.3 miR-31 促进结肠癌细胞集落形成能力

通过集落形成实验,观察 miR-31 对结肠癌细胞集落形成能力的影响。在 LoVo 细胞中,集落形成实验结果显示,过表达 miR-31 明显促进结肠癌细胞集落形成能力(Fig. 3A),敲减 miR-31 可以抑制结肠癌集落形成能力(Fig. 3B);在 HCT116 细胞中,也

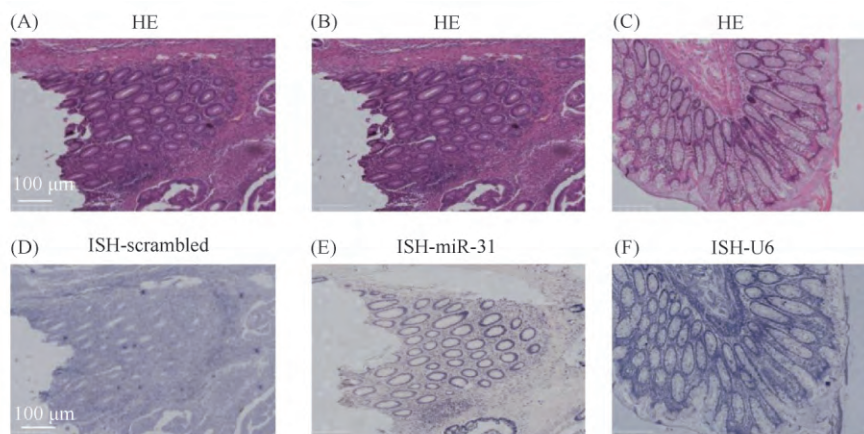


Fig. 1 miR-31 is increased *in situ* in colorectal cancer tissue The colorectal cancer samples were provided by Peking University Third Hospital and Peking University Health Science Center. The colorectal adenocarcinoma was diagnosed by HE staining. The tissue sections were dewaxed with xylene and alcohol and deproteinized using 0.2% glycine, 4% paraformaldehyde and proteinase K. The samples were pre-hybridized for 2 hours and incubated in the DIG-labeled miRCURYRNA probe for 16 hours. Then the samples were incubated in anti-DIG-AP Fab fragment overnight staining with NBT/BCIP for 48 hours. The pictures were taken with microscope after washing with PBS solutions. DIG-labeled miRCURYRNA probes included scramble probes (negative control), miR-31 probes and U6 probes (positive control). Blue-purple color indicates miR-31 expression. (A, B, C) HE staining of colorectal cancer samples; (D) *In situ* hybridization of scramble probes in colorectal cancer; (E) *In situ* hybridization of miR-31 probes in colorectal cancer; (F) *In situ* hybridization of U6 probes in colorectal cancer

观察到同样的现象(Fig. 3C, D)。随机选择 6 个视野进行统计。在 LoVo 细胞中, 过表达 miR-31 组, 每视野下的集落数是对照组的 2.2 ± 0.2 倍; 敲减 miR-31 组的集落数是对照组的 $37\% \pm 14\%$ (Fig. 3E, F); 在 HCT116 细胞中, 过表达 miR-31 组和敲减 miR-31 组的集落数分别是对照组的 3.3 ± 0.6 倍和 $35\% \pm 10\%$ (Fig. 3G, H)。

2.4 miR-31 靶向作用于 STK40 的 3'端非翻译区

通过数据库检索, 发现在 PicTar、TargetScan 和 miRanda 数据库预测的 miR-31 的众多作用靶点中, 丝氨酸/苏氨酸激酶 40 (serine/threonine kinase 40, STK40) 是三者共同预测的靶点, 而且目前在结肠癌中尚未有 STK40 的报道。为了验证上述数据库的预测结果, 在 HCT116 和 LoVo 细胞中, 分别转染 miR-31 mimics、inhibitor 以及相应的对照组。Western 印迹结果显示: 在 LoVo 细胞中转染 miR-31 mimics 后, 与对照组相比, STK40 蛋白表达量明显降低; 降低 miR-31 表达后, STK40 表达升高(Fig. 4A)。在 HCT116 细胞中也得到相似的趋势(Fig. 4B)。qRT-PCR 结果趋势与蛋白质结果趋势相同。结果显示, LoVo 细胞中, 转染 miR-31 mimics 后, LoVo 细胞 STK40 的 mRNA 水平降到对照组的 6%, miR-31 敲减后, 细胞 STK40 的 mRNA 水平为对照组的 3.5 倍

(Fig. 4C)。HCT116 细胞也得到相似的趋势。miR-31 过表达后, 细胞 STK40 的 mRNA 水平降到对照组的 39%; miR-31 敲减后, STK40 的 mRNA 水平为对照组的 2.1 倍(Fig. 4D)。双荧光素酶报告基因实验进一步证实, miR-31 可以结合 STK40 的 3'端非翻译区, 报告基因质粒 (STK40-3'-UTR vector) 和报告基因突变质粒 (STK40-3'-UTR mutation vector) 与 miR-31 共同转染 HEK-293T 细胞。结果显示: 与空载体报告基因组相比, STK40-3'-UTR 细胞中, 萤火虫荧光素酶活性下降为对照组 51%, 而 STK40-3'-UTR 突变后, 细胞中萤火虫荧光素酶活性变化不明显, 为对照组的 98%。上述实验结果证实, miR-31 可以通过结合 STK40-3'-UTR, 下调 STK40 mRNA 和蛋白质表达水平(Fig. 4E, 4F)。

2.5 miR-31 可促进 p65 核转位, 激活 NF- κ B 信号通路

STK40 被认为是 NF- κ B 信号通路的抑制因子。那么, miR-31 是否可以通过激活 NF- κ B 信号通路从而发挥促进结肠癌细胞增殖的作用呢? 免疫荧光结果显示, 在 HCT116 细胞中, 转染 miR-31 mimics 对照组 p65 蛋白基本定位于细胞浆内, 即 p65 蛋白处在未激活状态(Fig. 5A, B, C); 过表达 miR-31 后, p65 蛋白转位入核增加, 提示 NF- κ B 信号通路被激

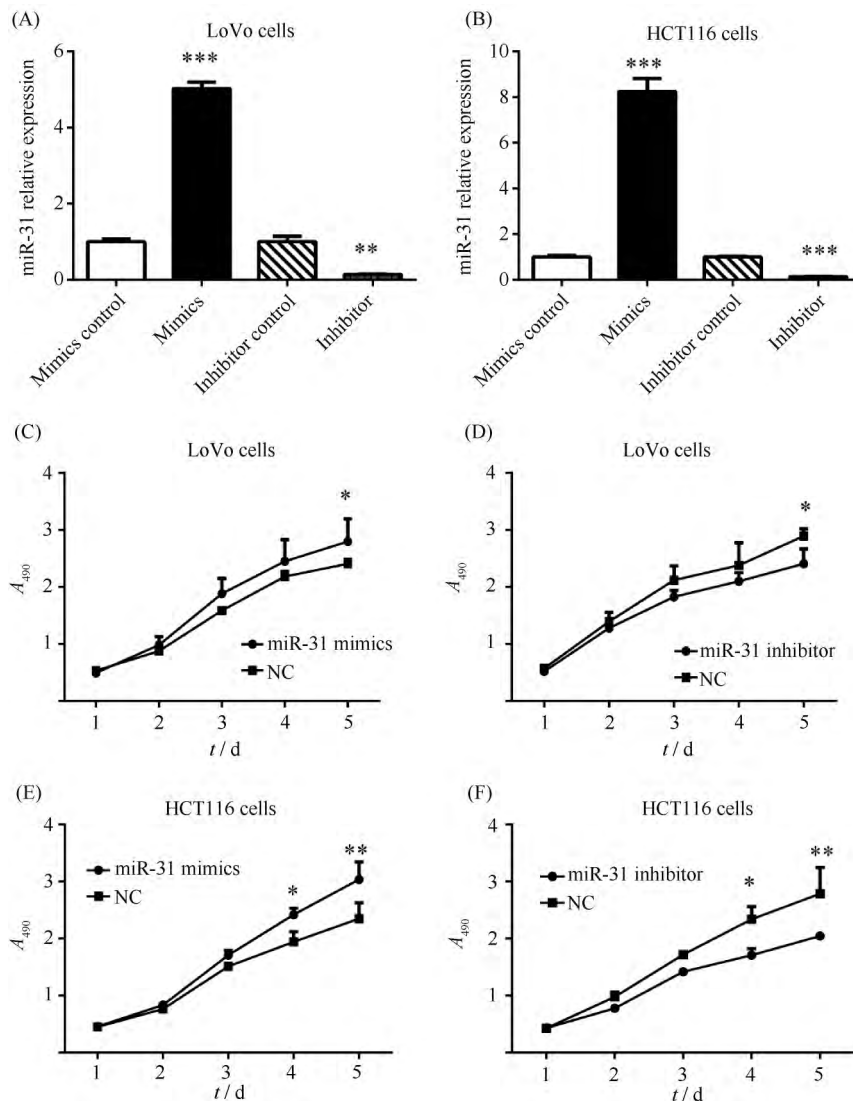


Fig. 2 miR-31 promotes the proliferation of colorectal cancer cells miR-31 mimics and inhibitor were transfected into colorectal cancer cells (HCT116 and LoVo cells). The cells were seeded in 96-well plates with 3 000 cells per well and 6 replicates in each group. The cells were incubated in medium with 20 μ L of MTS reagent at 37 $^{\circ}$ C for 3 hours. The absorbance values were measured at 490 nm wavelength. The assay was continued for 5 consecutive days and the proliferation curve was drawn. (A) The expression of miR-31 after miR-31mimics or inhibitor transfection in LoVo cells; (B) The expression of miR-31 after miR-31mimics or inhibitor transfection in HCT116 cells; (C) Proliferative curve after transfection of miR-31 mimics in LoVo cells; (D) Proliferative curve after transfection of miR-31 inhibitor in LoVo cells; (E) Proliferative curve after transfection of miR-31 mimics in HCT116 cells; (F) Proliferative curve after transfection of miR-31 mimics in HCT116 cells. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

活(Fig. 5D , E , F)。上述结果提示 miR-31 可以激活 NF- κ B 信号通路。

为了进一步证实 NF- κ B 信号通路上调, Western 印迹检测 miR-31 过表达或抑制后 p65 及其磷酸化 p65 的表达情况,以及 NF- κ B 信号通路中 cyclin D1 的表达。结果显示, miR-31 过表达后, 磷酸化 p65 表达增加, 反之, 抑制 miR-31 表达后, 磷酸化 p65 减少, 而细胞中总的 p65 蛋白变化并不明显(Fig. 5G)。密度扫描后, 磷酸化 p65 与 GAPDH 的比值分

别为 1.54 , 1.01 , 0.91 , 1.03。qRT-PCR 结果显示, miR-31 过表达可以上调 *cyclinD1* 表达, miR-31 过表达后, 细胞中的 *cyclinD1* mRNA 表达水平增加为对照组的 2.59 ± 0.46 倍; miR-31 表达降低后, *cyclinD1* 降为对照组的 $72.41\% \pm 12.85\%$ (Fig. 5H)。

2.6 miR-31 通过激活 NF- κ B 信号通路促进结肠癌细胞增殖

为了进一步验证 NF- κ B 在 miR-31 促进结肠癌细胞增殖中的作用, 在 HCT116 细胞中加入 NF- κ B

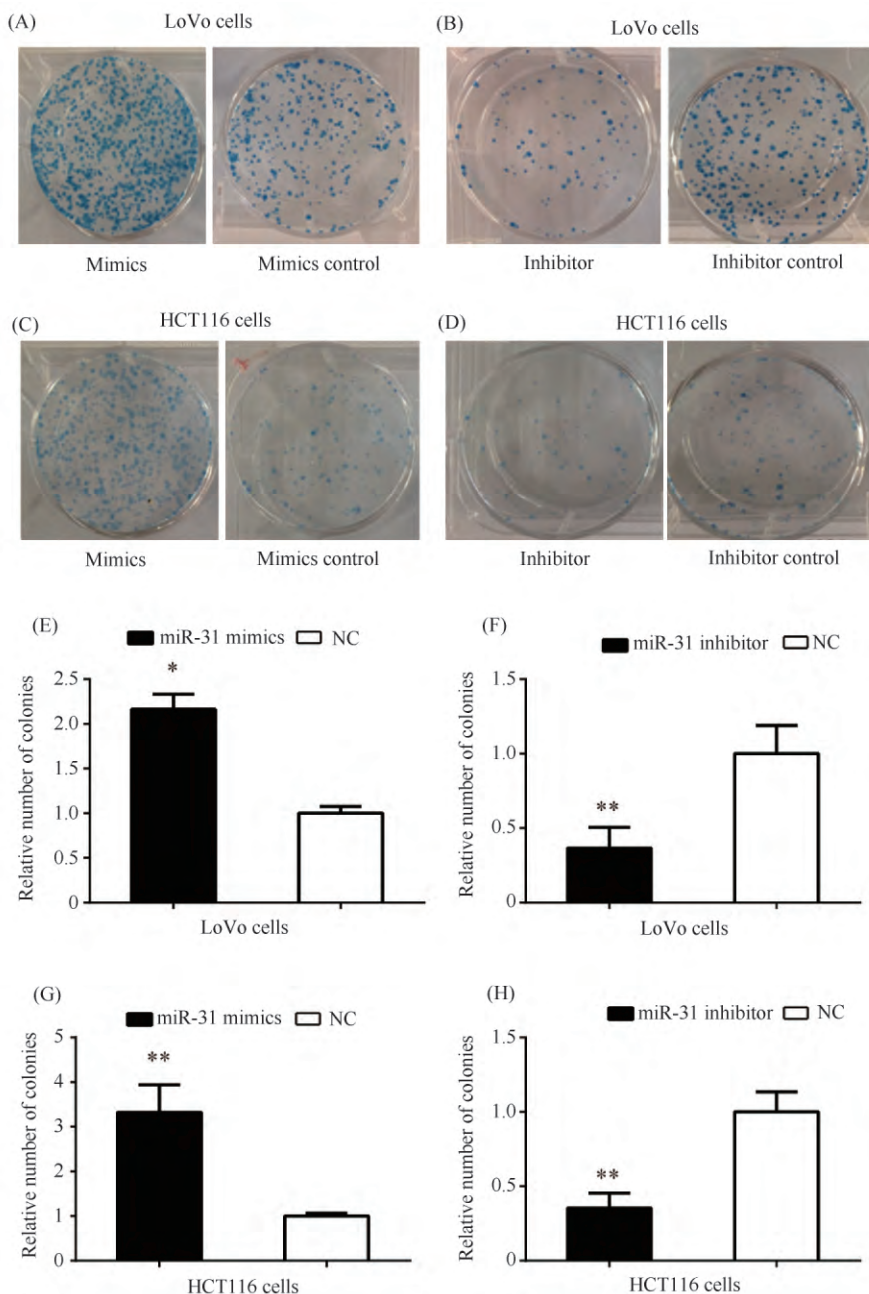


Fig. 3 miR-31 promotes the clonogenic ability of colorectal cancer cells The cells were seeded in 6-well plates with 9 000 cells per well at 37 °C for 10-14 days. After 10-14 days, the cells were fixed and stained with 0.1% methylene blue solution. The number of colonies was counted in random 6 fields. (A) Colony formation of LoVo cells after transfection of miR-31 mimics in LoVo cells; (B) Colony formation of LoVo cells after transfection of miR-31 inhibitor in LoVo cells; (C) Colony formation of HCT116 cells after transfection of miR-31 mimics in HCT116 cells; (D) Colony formation of HCT116 cells after transfection of miR-31 inhibitor in HCT116 cells; (E) The statistical results of colonies after transfection of miR-31 mimics in LoVo cells; (F) The statistical results of colonies after transfection of miR-31 inhibitor in LoVo cells; (G) The statistical results of colonies after transfection of miR-31 mimics in HCT116 cells; (H) The statistical results of colonies after transfection of miR-31 inhibitor in HCT116 cells. * $P < 0.05$; ** $P < 0.01$

抑制剂,观察 miR-31 对结肠癌细胞增殖的影响。增殖实验结果显示,与对照组相比,NF- κ B 抑制剂可以明显降低细胞增殖能力(Fig. 6A)。在过表达 miR-31 组和对照组中,同时加入 NF- κ B 抑制剂后,细胞

增殖实验结果显示,HCT116 细胞中,miR-31 过表达后与对照组之间的增殖能力并没有显著性差异(Fig. 6B)。这结果提示,NF- κ B 信号通路的激活可能是 miR-31 促进结肠癌细胞增殖的机制之一。

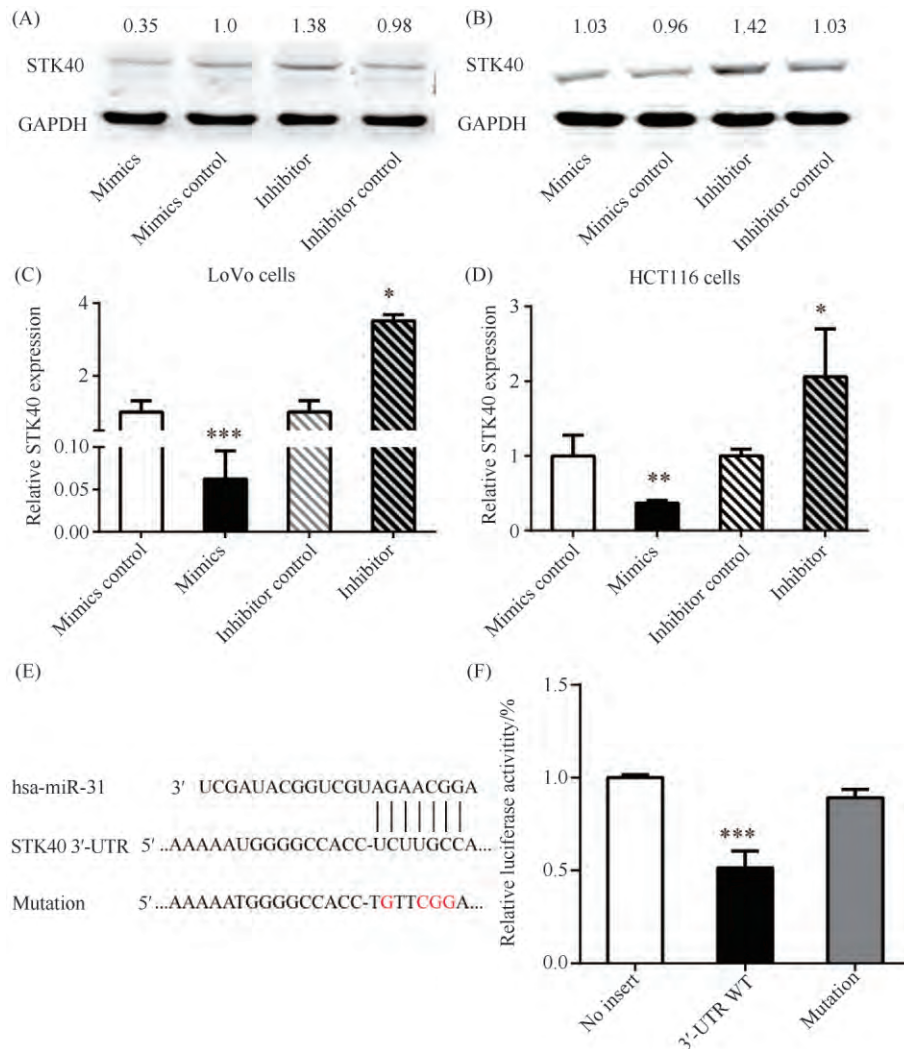


Fig. 4 miR-31 negatively regulates the expression of STK40 via targeting its 3'-UTR in CRC cells CRC cells (HCT116 and LoVo cells) were transfected with miR-31 mimics, inhibitor or the corresponding control for 48 hours. Cell lysates were prepared and analyzed by Western blotting for the expression of STK40. GAPDH was used as a loading control. Total RNAs were also isolated after the transfection. qRT-PCR for STK40 expression was performed. U6 was used as an internal control. The STK40-3'-UTR vector and STK40-3'-UTR mutation vector were further constructed, and were co-transfected with miR-31 into HEK-293T cells, respectively. Luciferase activities were measured by the Dual-Luciferase Reporter Assay System (Promega) after 48 hours. (A, B) The protein levels of STK40 after miR-31 mimics or inhibitor transfection measured by Western blotting; (C, D) The expression of STK40 after miR-31 mimics or inhibitor transfection measured by qRT-PCR; (E) The original sequence of STK40 3'-UTR (wt 3'-UTR) and the mutant sequence (mutation 3'-UTR) were shown and the red color indicated the mutation sites. (F) The transcriptional activity was measured by luciferase assay after co-transfection of miR-31 and STK40-3'-UTR vector or STK40-3'-UTR mutation vector. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

3 讨论

Tateishi 等发现, miR-31 促进结肠癌从早期腺瘤向癌的转化^[10]; Yang 等发现与结肠癌患者中 miR-31 表达高的患者相比, 表达低的患者预后更差, 高表达的 miR-31 是预后差的独立危险因素^[11]。本研究在此基础上, 通过原位杂交实验发现, miR-31 在结肠癌中的肿瘤细胞中原位表达增高, 结肠癌组织中的 miR-31 基本表达于肿瘤细胞内, 在癌旁组织中基本不表达。这进一步提示, miR-31 在结肠癌

的发生发展中, 可能发挥了重要作用。

其次, 体外实验证实, miR-31 促进结肠癌的细胞增殖和集落形成能力。Chen 等发现, miR-31 通过低氧诱导的转录因子 1α (hypoxia-inducible transcription factor 1α , HIF- 1α) 的抑制因子 1α (factor inhibiting HIF- 1α , FIH- 1α) 促进结肠癌增殖^[12]。Sun 等发现, miR-31 通过负调控 RASA1, 上调 RAS 信号通路, 从而促进结肠癌细胞增殖^[13]。上述结果与本文发现的结果一致。结肠癌中 miR-31 可以靶向调控多个基因。本研究通过生物信息

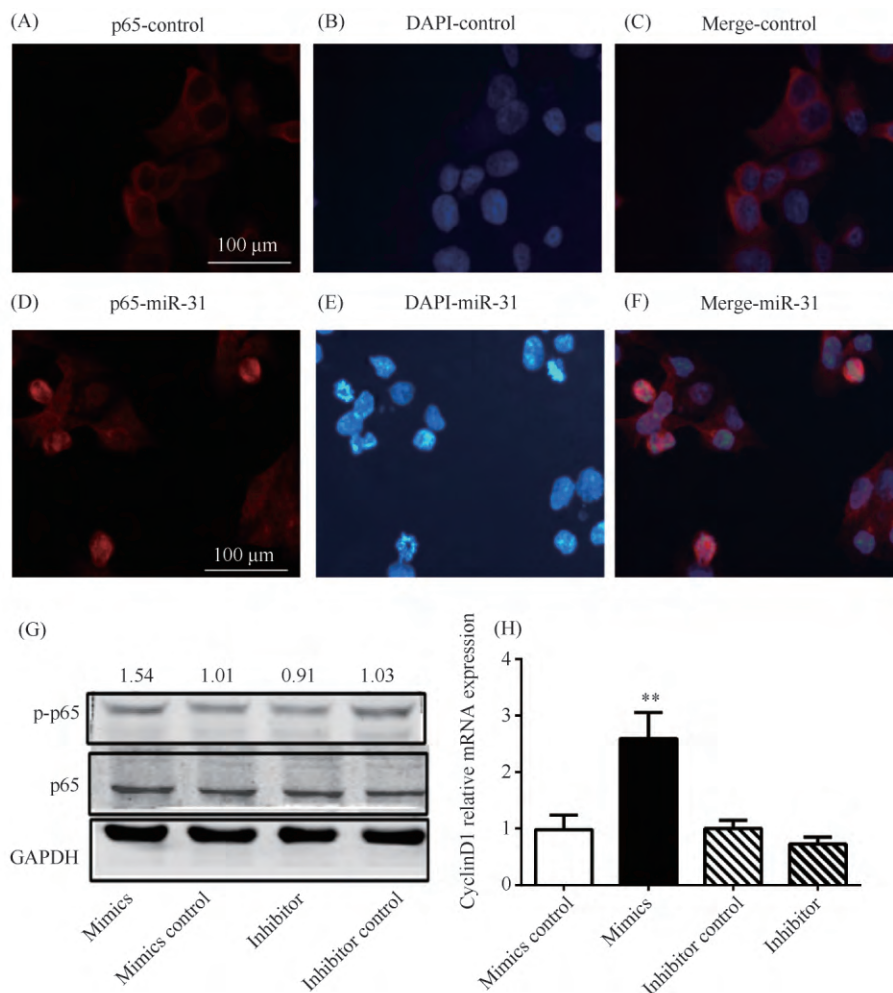


Fig. 5 miR-31 increases the translocation of phosphorylated p65 and activation of NF- κ B signal pathway in HCT116 cells

The cells transfected with miR-31 mimics, inhibitor and the corresponding control were seeded into a six-well plate dish and fixed with 4% paraformaldehyde. Incubation with fluorescence primary-antibody at 4 $^{\circ}$ C overnight, DAPI was stained for nuclei. The pictures were taken under the fluorescence microscope. Cell lysates were prepared and followed by Western blotting for the expression of phosphorylated p65 and total p65. GAPDH was used as a loading control. Total RNAs were also isolated for qRT-PCR to determine the expression in the indicated group. U6 was used as an internal control. (A, B, C, D, E, F) p65 nuclear translocation after miR-31 transfection in HCT116 cells; (G) The expression of phosphorylated p65 and total p65 after transfection of miR-31 mimics or inhibitor in HCT116 cell line; (H) The expression of *cyclin D1* after transfection of miR-31 mimics or inhibitor in HCT116 cell line. * $P < 0.05$; ** $P < 0.01$

学预测,结合分子生物学实验,发现在结肠癌细胞中,STK40(也称为SHIK/SgK495)是miR-31下游作用的靶基因之一。Huang等已经证实,STK40是NF- κ B信号通路的上游抑制因子^[14]。提示在结肠癌中,miR-31可能通过STK40影响其下游NF- κ B信号通路。而且,Taccioli等和Ning等已经发现,miR-31-STK40-NF- κ B信号通路在食管鳞状细胞癌(ESCC)的炎症-癌转换中发挥重要作用^[15,16]。在银屑病中也有研究报道,miR-31可以通过STK40上调NF- κ B信号通路,从而促进炎症反应^[17]。但是,目前尚未有结肠癌中STK40的报道。除此之外,miR-31在结肠癌中的靶基因还包括E2F2^[18]、FIH-1^[12]、

SATB1/2^[19]、RASA1^[13]和RhoBTB1^[20]等。因此,对其在结肠癌中的作用应该是多种靶点共同作用的结果。

最后,本研究发现,miR-31过表达后可以激活NF- κ B信号通路,使用NF- κ B抑制剂后,可以明显降低细胞增殖能力,提示NF- κ B信号通路的激活可能是miR-31促进结肠癌细胞增殖的机制之一。流行病学调查显示,结肠癌发病可能与结肠黏膜慢性炎症刺激有关,大约30%的慢性溃疡性结肠炎和克罗恩疾病会发展为结肠癌^[21]。在结肠癌中,NF- κ B通路往往是活化的。活化的NF- κ B在炎症与肿瘤发展关联中起着关键的积极作用^[22,23]。上述研究

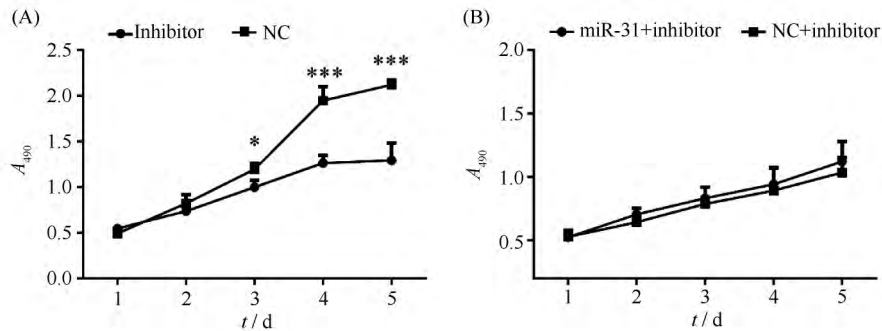


Fig. 6 miR-31 promotes the proliferation of colorectal cancer cells via activating NF- κ B signal pathway HCT116 cells transfected with miR-31 mimics were treated with NF- κ B inhibitor. MTS assay was then conducted. Cells were seeded in 96-well plates with 3 000 cells per well and 6 replicates in each group. The cells were incubated in medium with 20 μ L of MTS reagent at 37 $^{\circ}$ C for 3 hours, and measured at 490 nm wavelength. The assay was continued for 5 consecutive days. (A) Proliferative ability of miR-31 after inhibiting NF- κ B signal pathway in HCT116 cells; (B) Proliferative ability of miR-31 after inhibiting NF- κ B signal pathway in HCT116 cells. * $P < 0.05$; *** $P < 0.001$

也为本文提出的上调 NF- κ B 信号通路可能是 miR-31 促进结肠癌细胞增殖和形成能力的机制之一的假说提供了依据。

综上所述,本研究发现,miR-31 在结直肠癌中的肿瘤细胞中原位表达增高。体外实验证实,miR-31 促进结直肠癌细胞增殖,miR-31 靶向作用于 STK40,从而上调 NF- κ B 信号通路可能是其促增殖作用的机制之一。此外,除了其下游的靶基因之外,miR-31 受到哪些上游以及其他非编码 RNA (如 lncRNA, ceRNA 等) 的调节,并进而形成特定的调控网络等问题,也值得进一步探索。

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