

LncRNA NEAT1/miR-23b-3p/KLF3 轴调控结直肠癌细胞的生物学功能研究

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摘要: **目的** 探究长链非编码核糖核酸 (long non-coding RNAs, LncRNAs) NEAT1/miR-23b-3p/KLF3 调控轴对结直肠癌细胞生物学功能的影响。**方法** 利用癌症基因组图谱 (the cancer genome atlas, TCGA) 数据库分析结直肠癌组织和癌旁组织 NEAT1, miR-23b-3p 和 KLF3 表达水平, 并计算三者之间的相关性。以结直肠癌细胞系 (HT29 细胞) 作为实验对象, 实验被分为 Control 组 (空白对照组)、NC 组 (空载体组) 和 si-NEAT1 组 (NEAT1 干扰组)。CCK8 检测各组细胞的增殖情况, 流式细胞术检测各组细胞的凋亡、周期情况, Transwell 检测各组细胞的侵袭情况, 划痕实验检测各组细胞的迁移情况。在线工具 starbase 等预测能与 miR-23b-3p 靶向结合的基因, 采用人胚胎肾细胞 293 (human embryonic kidney cells 293, HEK293) 进行双荧光素酶报告基因实验验证。qRT-PCR 检测 NC 组、si-NEAT1 组、si-NEAT1+miR-23b-3p inhibitor 组 (共转染 si-NEAT1 和 miR-23b-3p inhibitor) 的 miR-23b-3p 和 KLF3 转录水平, 免疫印迹法检测以上三组 KLF3 蛋白质表达水平。**结果** 结直肠癌组织 NEAT1[5.29 (4.55,5.95)], KLF3[4.94 (4.62,5.24)] 表达高于癌旁组织 [4.79 (4.26,5.19), 4.49 (4.24,4.80)], 差异有统计学意义 ($U=6\ 677, P=0.001$; $U=28\ 257.5, P<0.001$), miR-23b-3p 表达低于癌旁组织 [9.99 (9.49,10.6) vs 10.80 (10.62,10.88)], 差异有统计学意义 ($U=2\ 906, P=0.004$)。结直肠癌组织中, NEAT1 和 KLF3 表达呈正相关 ($r=0.26, P<0.01$), miR-23b-3p 和 NEAT1, KLF3 表达量均呈负相关 ($r=-0.14, P=0.008$; $r=-0.17, P=0.001$)。与对照组相比, si-NEAT1 组使结直肠癌细胞增殖、迁移、侵袭的能力降低, 凋亡增加, 细胞被阻滞在 S 期, 结果差异均有统计学意义 (均 $P<0.05$)。starbase 预测 miR-23b-3p 靶基因为 NEAT1 和 KLF3。双荧光素酶报告基因实验也证实 miR-23b-3p 分子能互补结合 NEAT1 和 KLF3 3'UTR。与 NC 组相比, si-NEAT1 组 miR-23b-3p 表达上调, KLF3 转录和蛋白水平下调; 与 si-NEAT1 组相比, si-NEAT1+miR-23b-3p inhibitor 组 miR-23b-3p 表达下调, KLF3 转录和蛋白水平上调。**结论** NEAT1 可通过直接靶向 HT29 细胞中的 miR-23b-3p 上调 KLF3 表达, 增强结直肠癌细胞的增殖、侵袭和迁移等。

关键词: 结直肠癌; 长链非编码核糖核酸; 竞争性内源核糖核酸; 细胞增殖; 细胞迁移

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LncRNA NEAT1/miR-23b-3p/ KLF3 Axis Regulates the Biological Function of Colorectal Cancer Cells

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Abstract: Objective To explore the biological function of long non-coding RNAs (LncRNAs) NEAT1/miR-23b-3p/KLF3 regulatory axis in colorectal cancer. **Methods** The expression levels of NEAT1, miR-23b-3p and KLF3 in colorectal cancer tissues and adjacent tissues were analyzed by TCGA (the cancer genome atlas) database, and the correlations between the three molecular were calculated. HT29 cells were treated as the experimental object, which was divided into control group (blank control group), NC group (empty vector group) and si-NEAT1 group (NEAT1 interference group). CCK8 was used to detect cell proliferation, cell apoptosis and cell cycle were detected by flow cytometry, cell invasion was detected by Transwell, and cell

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migration was detected by wound-healing assay in each group. The online tool starbase etc. were used to predict the target genes of miR-23b-3p, and dual luciferase reporter gene assay was used to verify the target genes of miR-23b-3p by HEK293 (human embryonic kidney 293 cells). The transcript levels of miR-23b-3p and KLF3 in NC group, si-NEAT1 group and si-NEAT1+miR-23b-3p inhibitor group (co-transfected with si-NEAT1 and miR-23b-3p inhibitor) were detected by qRT-PCR, and KLF3 protein levels were detected by western blotting. **Results** NEAT1 [5.29 (4.55,5.95)] and KLF3 [4.94 (4.62,5.24)] levels were higher in colorectal cancer than in paracancerous tissues [4.79 (4.26,5.19), 4.49 (4.24,4.80)], the difference was statistically significant ($U=6\ 677$, $P=0.001$; $U=28\ 257.5$, $P<0.001$), and miR-23b-3p levels were lower in colorectal cancer than in paracancerous tissues [9.99 (9.49,10.6) vs 10.80 (10.62,10.88)], the difference was statistically significant ($U=2\ 906$, $P=0.004$), respectively. A positive correlation exists between NEAT1 and KLF3 expression levels ($r=0.26$, $P<0.01$), and expression of miR-23b-3p was inversely correlated with NEAT1 and KLF3 ($r=-0.14$, $P=0.008$; $r=-0.17$, $P=0.001$). Compared with control group, the ability of proliferation, migration and invasion was reduced, apoptosis increased, and cells were blocked in S phase in si-NEAT1 group for colorectal cancer cells, which were statistically significant (all $P<0.05$). The starbase, etc. predicted that the miR-23b-3p target genes were NEAT1 and KLF3. Dual luciferase reporter gene assay also confirmed that miR-23b-3p could bind NEAT1 and KLF3 3'UTR. In the si-NEAT1 group, the expression of miR-23b-3p was up-regulated, while the KLF3 transcript and protein levels were down-regulated through comparison with a NC group (all $P<0.05$). In the si-NEAT1+miR-23b-3p inhibitor group, the expression of miR-23b-3p was down-regulation, KLF3 transcript and protein levels were up-regulated through comparison with a si-NEAT1 group (all $P<0.05$). **Conclusion** NEAT1 can up-regulate the expression of KLF3 and enhance the proliferation, invasion, and migration of colorectal cancer cells by directly targeting miR-23b-3p in HT29 cells.

Keywords: colorectal cancer; long non-coding RNA; competitive endogenous RNA; cell proliferation; cell migration

结直肠癌 (colorectal cancer, CRC) 是一种高度恶性的肿瘤, 发病率和死亡率都在迅速上升, 发现时往往被诊断为临床晚期^[1-2]。因此, 积极寻求结直肠癌发病的调控基因和治疗靶点, 提升病人的生存时间和生活质量迫在眉睫。长链非编码核糖核酸 (long non-coding RNAs, lncRNAs) 是长度超过 200 个核苷酸的转录本, 没有或仅有有限的蛋白质编码功能^[3-4], 通过与 DNA, RNA 或蛋白质的相互作用参与多种致癌过程和疾病发生^[5]。lncRNAs 能通过影响癌症的功能特征 (比如不受控制的增殖、逃避细胞死亡以及转移形成), 或者直接 (间接) 地通过干扰不同的途径而发挥癌基因或肿瘤抑制因子的作用^[6-7]。在结直肠癌中, lncRNAs 还可通过多种调控方式影响结直肠癌细胞的生长, 并与结直肠癌的发生、转移和预后密切相关^[8]。SALMENA 等^[9]于 2011 年提出竞争性内源性核糖核酸 (competitive endogenous RNA, ceRNA) 假说, 认为 lncRNA, mRNA 或其他 RNA 分子, 可以作为天然 mRNA “海绵”, 通过共同的 miRNA 反应元件 (microRNA response elements, MREs) 与 miRNA 结合从而影响了 miRNA 导致的基因沉默。有研究显示, lncRNA NEAT1 在结直肠癌组织和细胞系中过表达, 参与肿瘤增殖、迁移、凋亡等^[11]。我们通过生物信息学工具成功预测 lncRNA NEAT1/miR23b-3p/KLF3 分子调控轴可能通过 ceRNA 参与调控结直肠癌发生。本研究最终以 HT29 细胞为实验对象, 初步验证该分子轴在结直肠癌的表达情况和调控机制, 以期对结直肠癌

提供新的策略和治疗靶点。

1 材料与方法

1.1 研究对象 HT29 (人结直肠癌细胞株) 和 HEK293 (人胚胎肾 293 细胞, 用于双荧光基因转染) 购自中国科学院上海细胞库。

1.2 仪器与试剂 McCoy's 5A 完全培养液 (凯基生物, 货号 KGM4892S); CCK-8 法细胞增殖检测试剂盒 (凯基生物, 货号 KGA317); Annexin V-APC/7-AAD apoptosis kit Cell Cycle Staining Kit (联科生物); Trizol 试剂 (康为世纪); qRT-PCR 试剂 (生命互联); 双荧光素酶报告基因检测试剂盒 (美国 Promega 公司); KLF3 抗体 (Cell Signaling Technology 公司), 二抗 IgG (北京康维公司)。

1.3 方法

1.3.1 生物信息学分析: 在线工具 starbase (<http://starbase.sysu.edu.cn/index.php>) 预测 miRNA 和 lncRNA 之间的互作关系。利用多个在线工具 (PITA, RNA22, miRmap, microT, miRanda, PicTar 和 TargetScan) 预测 miRNA-mRNA 之间的互作关系。最终预测 NEAT1/miR-23b-3p/KLF3 调控轴为研究对象。癌症基因组图谱 (the cancer genome atlas, TCGA) 数据库分析 NEAT, miR-23b-3p, KLF3 在结直肠癌组织及癌旁组织中的表达水平及其相关性。

1.3.2 细胞培养与转染: HT29 细胞均置于含 10g/dl 胎牛血清的 McCoy's 5A 培养液, 37℃ 和 5ml/dl CO₂ 培养箱中培养。实验分为 Control 组、NC 组和 si-NEAT1 组 (取干扰效果最好的一组)。转染前

24h, 将生长良好的细胞铺于6孔板上, 细胞密度约 5×10^5 /孔, 待细胞达到70%~90%融合时, 按Lipofectamine 2000转染试剂说明书对HT29细胞进行转染。

1.3.3 CCK8检测细胞增殖活性: 将转染后的细胞消化、重悬和计数, 并向96孔板加入100 μ l细胞悬液(约7000个/孔); 培养24h后使细胞贴壁, 再加入10 μ l CCK8试剂, 置于培养箱中孵育2h, 450nm波长, 酶标仪(北京六一生物科技, 型号TD4A)检测每孔的吸光值。

1.3.4 流式细胞术检测细胞凋亡: 调整HT29细胞浓度为 1×10^5 个/ml, 加1ml PBS 1500 r/min离心3 min, 洗两遍。取300 μ l预冷的1 \times Binding Buffer重悬细胞, 每管加入3 μ l Annexin V-APC和5 μ l 7-AAD。轻微混匀后, 室温避光孵育10 min。再向每管中加入200 μ l预冷的1 \times Binding Buffer, 混匀后流式仪检测。

1.3.5 流式细胞术检测细胞周期: 将细胞悬液1500 r/min离心3 min, 弃上清。加入1ml PBS, 1500 r/min离心弃上清, 加1ml DNA Staining solution和10 μ l Permeabilization solution, 涡旋振荡5~10s混匀, 室温避光孵育30min, 流式细胞仪检测(NovoCyte 2060R, 杭州艾森生物公司)。

1.3.6 Transwell检测细胞侵袭能力: 转染后的细胞消化收集, 1500 r/min离心10min, 细胞浓度调至 1×10^5 /ml。上室加入100 μ l无血清的细胞悬液, 下室(侵袭小室)加入500 μ l 10g/dl胎牛血清培养液。随后放入37℃含5ml/dl CO₂的培养箱中培养24h, 取出小室, PBS洗涤3次。室温下, 小室放在95%乙醇中固定5 min; 0.5g/dl结晶紫染色10 min, PBS漂洗, 用棉签轻轻拭去小室滤膜上层细胞, 显微镜下观察结果。

1.3.7 划痕实验检测细胞迁移能力: 将细胞铺板待密度达到90%以上后进行划线, 使用200 μ l枪头在每孔进行划痕, 弃去培养基, PBS清洗3次, 换成无血清培养基后给每孔划痕拍照。将细胞放入培养箱中, 24h后再次给每孔划痕拍照。

1.3.8 蛋白质印记法检测KLF3蛋白的表达: 使用细胞裂解液裂解各组细胞样本, 4℃12000r/min离心10min后(离心半径6.26cm), 获得蛋白样品, 采用考马斯亮蓝测量蛋白质浓度。取相应体积的蛋白并加入上样缓冲液, 混匀后, 沸水浴加热3min, 使蛋白变性。采用10g/dl SDS-PAGE凝胶进行蛋白分离, 电泳先用80V, 待溴酚蓝进入分离胶后改用120V, 电泳1~2h。在冰浴中进行转膜, 转膜电流为300mA, 时间为60 min。转膜后把膜放入洗涤液中漂洗1~2 min, 再将膜放入封闭液中室温

封闭60 min。一抗(1:1000)4℃过夜, 二抗室温下孵育2~3h, 显影检测蛋白表达。

1.3.9 荧光定量PCR检测各分组基因表达: TRIzol法提取各组总RNA, 紫外分光光度法检测RNA的纯度和浓度。通过逆转录合成cDNA, 以cDNA为模板, 以GAPDH或U6为内参, 每个基因扩增设置3个复孔。反应条件: 95℃预变性10min; 95℃变性10s, 56℃退火30s, 72℃延伸30s, 共计40个循环, 伯乐荧光PCR仪上分析(SYBR Green荧光染料法)。LncRNA NEAT1正向引物序列: 5'-GTTTGCCTGCCTTCTTGTC-3', 反向引物序列: 5'-TACCCTCCCAGCGTTTAGC-3'; miR-23b-3p正向引物序列: 5'-CGATCACATTGCCAGGGA T-3', 反向引物序列: 5'-AGTGCAGGGTCCGAGG TATT-3'; KLF3正向引物序列: 5'-GAGGCGATCG CCATGCTCATGTTTGACCCAGTTC-3', 反向引物序列: 5'-GCGACGCGTTCAGACTAGCATGTGGCG TTTC-3'; GAPDH正向引物序列: 5'-TGACTTC AACAGCGACACCCA-3', 反向引物序列: 5'-CAC CCTGTTGCTGTAGCCAAA-3'; U6正向引物序列: 5'-CTCGCTTCGGCAGCACA-3', 反向引物序列: 5'-AACGCTTCACGAATTTGCGT-3'。基因相对表达量以 $2^{-\Delta\Delta Ct}$ ($\Delta\Delta Ct = \Delta Ct_{\text{试验}} - \Delta Ct_{\text{对照}}$) 法计算。

1.3.10 双荧光素酶报告基因检测: 将含有预测的miR-23b-3p靶位点3'-UTR的野生型(wt)或突变型(mut)NEAT1, KLF3基因克隆到pGL3载体(Promega公司提供, 上海)。再使用Lipofectamine 2000将构建好的载体与mimics miR-23b-3p共转染HEK293细胞, 每组重复3次; 转染48h后, 双荧光素酶检测试剂盒(Promega)用于分析荧光素酶活性。

1.4 统计学分析 采用GraphPad Prism 8.0软件进行统计分析。如数据呈正态分布, 定量结果采用均数 \pm 标准差($\bar{x} \pm s$)表示。如数据呈非正态分布, 采用中位数(四分位数)[M(P₂₅, P₇₅)]表示。两组之间定量数值比较采用独立样本t检验或Mann-Whitney U检验。多组之间定量数值比较采用单因素方差分析, 组间两两比较采用LSD-t检验, 检验水准 $\alpha=0.05$, 以 $P < 0.05$ 为差异具有统计学意义。生物信息学分析采用R软件或在线工具完成。

2 结果

2.1 结直肠癌组织和癌旁组织NEAT1, miR-23b-3p, KLF3的表达及三者相关性比较 见表1。TCGA数据库分析得知, NEAT1和KLF3在结直肠癌组织中表达显著高于癌旁组织, miR-23b-3p在癌组织中的表达低于癌旁组织, 差异具有统计学意义(均 $P < 0.05$)。其中, NEAT1与KLF3表达呈显著正

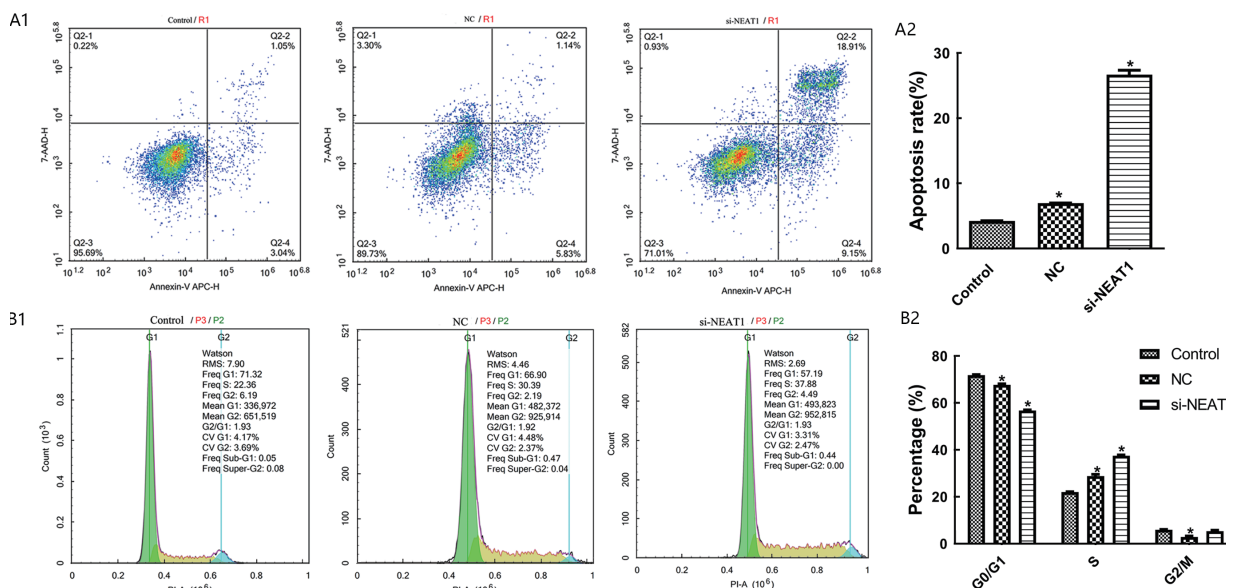
相关 ($r=0.26, P<0.01$), miR-23b-3p 与 NEAT1, KLF3 表达量均呈显著负相关 ($r=-0.14, P=0.008$; $r=-0.17, P=0.001$)。

表 1 结直肠癌组织和癌旁组织 NEAT1, miR-23b-3p, KLF3 的表达

项目	癌旁组织		癌组织		U	P
	n	M(P ₂₅ , P ₇₅)	n	M(P ₂₅ , P ₇₅)		
NEAT1	41	4.79 (4.36, 5.19)	480	5.29 (4.55, 5.95)	6677	0.001
miR-23b-3p	8	10.80 (10.62, 10.88)	457	9.96 (9.49, 10.6)	2906	0.004
KLF3	349	4.49 (4.24, 4.80)	290	4.94 (4.62, 5.24)	28257.5	<0.001

注: NEAT1 和 KLF3 表达量单位为 $\log_2(\text{FPKM}+1)$; miR-23b-3p 表达量单位为 $\log_2(\text{RPM}+1)$ 。

2.2 干扰 NEAT1 表达 (siNEAT1 组) 对 HT29 细胞增殖、凋亡、细胞周期、迁移和侵袭的影响 见表 2。si-NEAT1 组的凋亡率显著高于 Control 组 ($P<0.05$), 见图 1A1&A2。siNEAT1 组 G0/G1 期细胞比例明显少于对照组 ($P<0.001$), 见图 1B1 &B2。siNEAT1 组 S 期细胞比例显著高于 Control 组 ($P<0.001$)。siNEAT1 组细胞明显的被阻滞在 S 期。siNEAT1 组的侵袭能力明显低于 Control 组和 NC 组 ($t=8.33, 6.54$, 均 $P<0.01$), siNEAT1 组的增殖能力明显低于 Control 组和 NC 组 ($t=5.17, 2.92$, 均 $P<0.01$), siNEAT1 组的迁移能力明显低于 Control 组 ($U=0, P<0.01$)。



注: A1&A2: si-NEAT1 对 HT29 细胞凋亡的影响; B1&B2: si-NEAT1 对 HT29 细胞周期的影响; * 代表与对照组相比 $P<0.05$, 每组实验重复 3 次。

图 1 siNEAT1 对 HT29 细胞凋亡和周期的影响

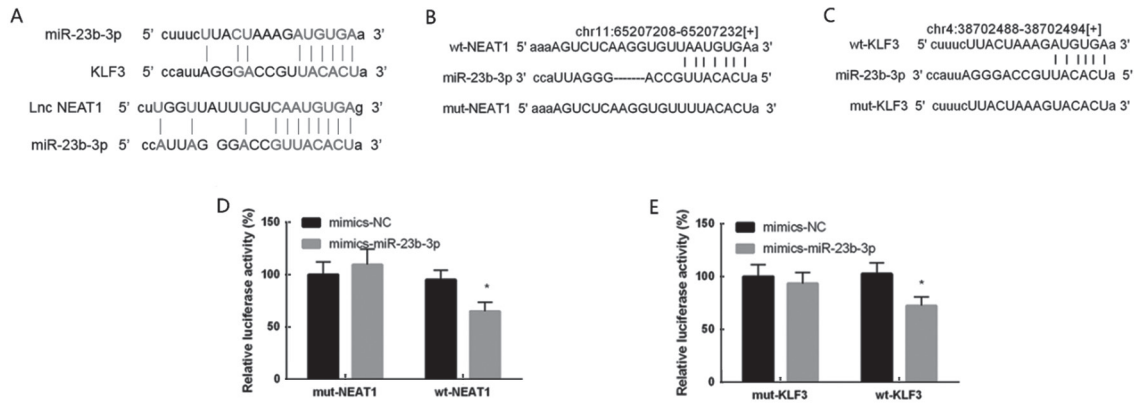
表 2 siNEAT1 对 HT29 细胞侵袭、增殖和迁移的影响

类别	Control 组	NC 组	siNEAT1 组	F	P
侵袭细胞数	75 ± 9	67 ± 10	23 ± 6	54.12	<0.001
细胞增殖 A 值	100.00 ± 5.02	90.64 ± 4.36	80.34 ± 4.27	23.24	<0.01
细胞迁移速率 (μm/h)	3.06 ± 0.40	1.56 ± 0.66	1.31 ± 1.16	21.43	<0.001

2.3 miR23b-3p 潜在靶点的预测和验证 见图 2。在线工具 STARBASE 等预测 miR-23b-3p 可同时与 NEAT1 和 KLF3 3'UTR 互补配对 (图 2A)。双荧光素酶报告基因实验显示, 插入 wt-NEAT1 或 wt-KLF3 序列的载体与 mimics miR-23b-3p 共转染 HEK293 细胞后, 荧光素酶活性显著降低; 而插入 mut-NEAT1 或 mut-KLF3 序列的载体与 mimics miR-23b-3p 共转染 HEK293T 细胞后, 荧光素酶活性没有明显变化, 验证了 NEAT1 和 KLF3 与 miR-23b-3p 的靶向结合 (图 2B~E)。

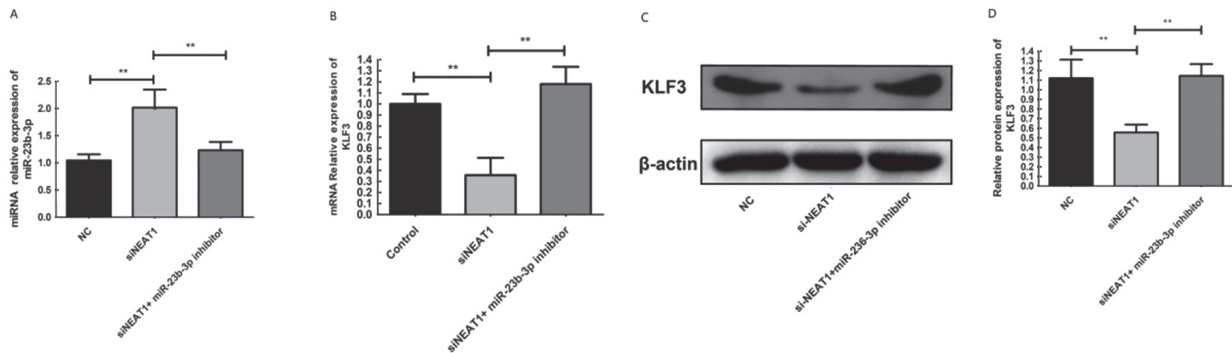
2.4 NEAT1 通过 miR-23b-3p 对 KLF3 表达的影响 见图 3。与 NC 组相比, siNEAT1 组的 miR-23b-3p 表达显著上调 (2.02 ± 0.33 vs 1.05 ± 0.11), KLF3 转录 (0.36 ± 0.16 vs 1.00 ± 0.09) 和蛋白 (0.56 ± 0.08 vs 1.12 ± 0.19) 表达显著下降, 差异均有统计学意义 ($t=4.840, 6.038, 1.704$, 均 $P<0.05$)。与 si-NEAT1 组相比, si-NEAT1+miR-23b-3p inhibitor 组 miR-23b-3p 表达下调 (1.23 ± 0.15 vs 2.02 ± 0.33), KLF3 转录 (1.18 ± 0.16 vs 0.36 ± 0.16) 和蛋白 (1.14 ± 0.12 vs 0.56 ± 0.08) 水平上调, 差异均有统计学意义。

($t=3.774\ 8, 6.276\ 8, 6.965\ 6$, 均 $P<0.05$)。



A: 在线工具 starbase 等预测 miRNA - 23b - 3p 与靶基因 NEAT1 和 KLF3 基因序列的关系; B&C: NEAT1 与 miR-23b-3p 以及 KLF3 与 miR-23b-3p 结合位点的野生序列和突变序列; D&E: 插入 mut-NEAT1 或 mut-KLF3 序列的载体与 mimics miR-23b-3p 共转染 HEK-293 细胞后, 荧光素酶活性变化。注: * $P<0.05$, 每组实验重复 3 次。

图2 miR-23b-3p 潜在靶点的预测和验证



A. HT29 细胞转染 NC, si-NEAT1 或 si-NEAT1+miR-23b-3p inhibitor 后 miR-23b-3p 转录水平; B. KLF3 转录水平; C~D. KLF3 蛋白水平变化。注: * $P<0.05$, 每组实验重复 3 次。

图3 NEAT1 通过 miR-23b-3p 对 KLF3 表达的影响

3 讨论

NEAT1 已被证实存在于乳腺癌、肝癌、肺癌、结直肠癌、卵巢癌、胶质瘤等发挥着重要的致癌作用, 且多与肿瘤的预后不良有关^[10-14]。乳腺癌的体内动物实验证实, NEAT1 沉默能有效降低肿瘤生长并能显著提升肿瘤对化疗药物顺铂的敏感性^[15]。卵巢癌中, 敲降 NEAT1 基因表达能通过调控 miR-770-5p/PARP1 轴显著抑制顺铂的耐药性^[16]。NEAT1 还可通过 WNT/ β -catenin 信号通路促进恶性胶质瘤细胞增殖和肿瘤进展^[17]。本研究表明, NEAT1 在结直肠癌中表达上调, 并且 NEAT1 作为致癌基因促进细胞增殖、迁移、侵袭和抑制细胞凋亡。双荧光素酶实验证实, 在体外结直肠癌细胞中, miR-23b-3p 分子可特异结合 NEAT1 和 KLF3 分子。因此, 探讨 NEAT1/miR-23b-3p/KLF3 轴在结直肠癌中如何发挥作用具有重要意义。

在结直肠癌组织中, 有研究者利用微阵列芯片基因分析法和 RT-PCR 检测, 不断发现异常表达的 miRNA, miRNA 也因此被作为结直肠癌的潜在生物标志物^[18-19]。miR-23b-3p 可通过下调 ZNF281 来提

升结直肠癌对 5'FU 化疗的敏感性, 且其在多种肿瘤中扮演着抗癌角色^[20]。又有研究表明 miR-23b-3p 在结直肠癌细胞系中显著下调, 并能抑制细胞的迁移和侵袭^[21-23], 这和本文的研究结果一致, 可能进一步证实其在结直肠癌中的肿瘤抑制作用。本研究证实 miR-23b-3p 被 NEAT1 分子负向调控, 这主要是其通过吸附 miR-23b-3p 分子而实现的。国内外多项研究证明, miR-23b-3p 被认为是多种肿瘤细胞生理中重要的调控分子。miR-23b-3p 可以通过靶向结合多个下游基因分子 (FZD7, PLA2, PTEN 等) 调控肿瘤的发生发展^[24]。miR-23b-3p 可能参与多种肿瘤发病机制的调控, 如通过 miR-23b-3p/MRC2 轴调控卵巢癌的预后发展^[25], miR-23b-3p 的过表达可能抑制人宫颈癌 CasKi 细胞的增殖、迁移、侵袭和上皮-间质转化过程^[26]。这些结果均有利的证明 miR-23b-3p 在结直肠癌和其他肿瘤中发挥着重要的调控作用。

据我们所知, KLF3 在肺癌、黑色素瘤、胰腺癌等^[27-29]多种肿瘤中发挥着致癌基因的作用, 但是其在结直肠癌中的表达情况却鲜见报道。生物

信息学分析表明, KLF3 在结直肠癌中高表达, 且和 NEAT1 呈显著正相关。miR-23b-3p 和 NEAT1, KLF3 均呈负相关性 ($P<0.05$), NEAT1 和 KLF3 呈显著正相关 ($P<0.05$)。这些结果有利于证明 NEAT1/miR-23b-3p/KLF3 轴在结直肠癌中可能发挥调控作用。体外生物细胞学功能研究进一步在结直肠癌细胞系 HT29 中证实 miR-23b-3p 能够靶向结合下游基因 KLF3, KLF3 转录和蛋白水平表达又受到 NEAT1 和 miR-23b-3p 分子的精确调控, 这可能说明 KLF3 在结直肠癌中也是一种致癌基因, NEAT1/miR-23b-3p/KLF3 轴在结直肠癌中可能发挥关键作用, NEAT1 可通过靶向结合 miR-23b-3p 来上调 KLF3 表达。但这些仍需要更加详尽的生物学实验去证实。

诚然, 本研究也存在一定的不足。首先, 仍然需要体内实验进一步证明 NEAT1/miR-23b-3p/KLF3 的作用。其次, 本研究仅选用一种结直肠癌细胞系进行研究, 且未对 KLF3 分子做深入细致的机制研究等。总之, 我们通过体外研究对 NEAT1 基因进行一定的功能验证, 初步证实了其在结直肠癌中调节细胞增殖、迁移、侵袭和细胞凋亡的功能, 鉴定了一种新的 NEAT1/miR-23b-3p/KLF3 调控轴, 可能对结直肠癌发病机制和潜在靶点研究提供新的思路和策略。

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