

circ_0015756通过调控miR-515-5p/HMGB3轴影响肺癌细胞增殖、凋亡和迁移

许俊¹ 朱承莹¹ 王德钧^{2*}

¹东南大学附属中大医院溧水分院, 南京市溧水人民医院呼吸内科, 南京 211200;

²江苏省中医院呼吸与危重病医学科, 南京 210029)

摘要 为探讨环状RNA 0015756(circ_0015756)对肺癌细胞增殖、凋亡和迁移的影响和潜在机制, 该研究采用实时定量PCR(RT-qPCR)分析肺癌组织和癌旁组织中circ_0015756和微小RNA(miR)-515-5p的表达水平。同时, 将circ_0015756小干扰RNA(si-circ_0015756)、miR-515-5p模拟物、si-circ_0015756+miR-515-5p抑制物分别转染肺癌细胞A549, 采用四甲基偶氮唑蓝实验、平板克隆实验检测A549细胞的增殖能力, 采用流式细胞术分析A549细胞的凋亡率, 采用划痕愈合实验和Transwell实验检测A549细胞的迁移能力。蛋白质印迹法测定高迁移率族蛋白3(HMGB3)的表达水平。双荧光素酶分析circ_0015756与miR-515-5p、miR-515-5p与HMGB3的靶向关系。结果显示, 肺癌组织中circ_0015756的相对水平显著高于癌旁组织($P < 0.05$), miR-515-5p的相对水平显著低于癌旁组织($P < 0.05$)。干扰circ_0015756表达后A549细胞增殖抑制率、凋亡率、miR-515-5p的相对水平显著升高($P < 0.05$), 集落形成数、迁移距离、迁移细胞数、HMGB3蛋白的相对水平显著降低($P < 0.05$)。过表达miR-515-5p后A549细胞增殖抑制率、凋亡率显著升高($P < 0.05$), 集落形成数、迁移距离、迁移细胞数、HMGB3蛋白的相对水平显著降低($P < 0.05$)。抑制miR-515-5p表达明显减弱干扰circ_0015756表达对A549细胞增殖、集落形成、迁移以及HMGB3蛋白表达的影响($P < 0.05$)。circ_0015756与miR-515-5p直接结合, miR-515-5p与HMGB3直接结合。总之, 干扰circ_0015756通过靶向上调miR-515-5p/HMGB3轴抑制肺癌细胞增殖和迁移, 诱导细胞凋亡。

关键词 circ_0015756; 肺癌; 细胞增殖; 迁移; 凋亡; miR-515-5p; 高迁移率族蛋白3

circ_0015756 Affects Cell Proliferation, Apoptosis and Migration by Regulating miR-515-5p/HMGB3 Axis in Lung Cancer

XU Jun¹, ZHU Chengying¹, WANG Dejun^{2*}

¹Department of Respiratory Medicine, Zhongda Hospital Lishui Branch, Southeast University;

Nanjing Lishui People's Hospital, Nanjing 211200, China; ²Department of Respiratory and Critical Care Medicine, Jiangsu Provincial Hospital of Traditional Chinese Medicine, Nanjing 210029, China)

Abstract In order to investigate the effect of circ_0015756 (circular RNA 0015756) on the proliferation, apoptosis and migration of lung cancer cells and its underlying mechanism, RT-qPCR was applied to analyze the expression of circ_0015756 and microRNA (miR)-515-5p in lung cancer tissues and adjacent tissues. Meanwhile, si-circ_0015756 (circ_0015756 small interfering RNA), miR-515-5p mimic, si-circ_0015756+miR-515-5p inhibi-

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*通讯作者。Tel: 13701467821, E-mail: lsjwdj@sina.com

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*Corresponding author. Tel: +86-13701467821, E-mail: lsjwdj@sina.com

tor were transfected into lung cancer cell A549, respectively. The proliferation ability of A549 was assessed by methyl thiazolyl tetrazolium method and plate cloning assay. The apoptosis rate of A549 was measured by flow cytometry, and the migration ability of A549 was evaluated by scratch healing and Transwell test. Western blot was used to assess the expression level of HMGB3 (high mobility group protein 3). The targeting relationships between circ_0015756 and miR-515-5p, miR-515-5p and HMGB3 were verified by dual luciferase assays. The results showed that the relative level of circ_0015756 in lung cancer tissue was significantly higher than that in adjacent tissues ($P<0.05$), while the relative level of miR-515-5p was significantly lower than that in adjacent tissues ($P<0.05$). After interfering with the expression of circ_0015756, the cell proliferation inhibition rate, apoptosis rate, and miR-515-5p relative levels of A549 cells were significantly increased ($P<0.05$), and the colony forming numbers, migration distance, migration cell numbers and relative levels of HMGB3 protein were significantly reduced ($P<0.05$). After overexpression of miR-515-5p, the proliferation inhibition rate and apoptosis rate of A549 cells were significantly increased ($P<0.05$). The number of colonies formed, migration distance, migration cell numbers and the relative level of HMGB3 protein were significantly reduced ($P<0.05$). miR-515-5p inhibition significantly reduced the effect of interfering with circ_0015756 on the proliferation, colony formation, migration and HMGB3 protein expression of A549 cells ($P<0.05$). circ_0015756 directly bound to miR-515-5p, and miR-515-5p directly bound to HMGB3. In conclusion, interference circ_0015756 inhibited the proliferation and migration, and induced cell apoptosis of lung cancer cells by targeting and up-regulating miR-515-5p/HMGB3 axis.

Keywords circ_0015756; lung cancer; cell proliferation; migration; apoptosis; miR-515-5p; high mobility group protein 3

肺癌是危害人类健康的主要疾病之一,全球每年新增病例超过180万例^[1]。虽然包括手术、放疗和分子靶向治疗在内的肺癌治疗策略不断进展,但5年生存率仍较低^[2]。远端转移是导致肺癌治疗失败的主要因素,然而肺癌转移影响因素复杂,其潜在机制并未阐明。因此,确定肺癌转移的关键机制对开发有效的肺癌治疗策略至关重要。环状RNA(circular RNA, circRNA)是具有共价闭环结构的新型内源性RNA,研究证实circRNA具有组织表达特异性,并通过靶向微小RNA(microRNA, miRNA)间接调控mRNA表达来参与肿瘤的发生和发展,是癌症治疗的潜在靶点^[3]。研究表明,肝癌组织中circRNA 0015756(circ_0015756)表达上调,敲减circ_0015756明显降低肝癌细胞的增殖、侵袭、迁移和对凋亡的抵抗能力^[4-5]。然而circ_0015756在肺癌中生物学功能和潜在机制尚不清楚。研究报道,肺癌细胞中miR-515-5p表达降低,过表达miR-515-5p可抑制肺癌细胞存活和转移。高迁移率族蛋白3(high mobility group protein 3, HMGB3)高表达与肺癌的恶性进展和不良预后有关,敲减HMGB3对肺癌细胞具有促凋亡和抗增殖作用^[6-7]。靶基因预测到miR-515-5p是circ_0015756的潜在靶点, HMGB3是

miR-515-5p的潜在靶点,因此,本研究基于circRNA-miRNA-mRNA调控网络探讨circ_0015756在肺癌进展中的作用,旨在为肺癌治疗提供有效靶点。

1 材料与方法

1.1 实验材料

1.1.1 组织来源 收集35例(2017年6月至2020年12月间在南京市溧水人民医院收治)原发性肺癌患者的肺癌组织和癌旁组织标本(距离原发病灶5 cm,无肿瘤细胞浸润)。男性19例,女性16例,年龄在54~75岁。手术切除后,将标本快速置于液氮中冷冻,然后转移到-80 °C的冰箱中保存。本研究经南京市溧水人民医院医学伦理委员会批准,所有患者均签署知情同意书。纳入标准:术前未接受化疗、放疗等抗肿瘤治疗;术后病理学证实为肺癌。排除标准:术前接受抗肿瘤治疗患者;罹患其他恶性肿瘤患者。

1.1.2 细胞和试剂 人肺癌细胞A549、人正常肺上皮细胞BEAS-2B购自中国科学院细胞库;反转录试剂盒、SYBR Premix Ex Taq、Trizol试剂购自大连宝生物工程公司;miR-515-5p模拟物及其对照(miR-NC)、circ_0015756的小干扰RNA(si-circ_0015756)及其阴性对照(si-NC)、miR-515-

5p的抑制物(anti-miR-515-5p)、荧光素酶报告质粒购自上海吉玛制药技术有限公司;电化学发光(electrochemiluminescence, ECL)试剂盒、膜联蛋白V(Annexin V)-异硫氰酸荧光素(fluorescein isothiocyanate, FITC)-碘化丙啶(propidium iodide, PI)凋亡检测试剂盒、结晶紫染色液购于北京索莱宝科技有限公司;鼠源HMGB3单克隆抗体(M02834)、鼠源甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase, GAPDH)单克隆抗体(BM1623)购于武汉博士德生物工程有限公司;四甲基偶氮唑蓝(methyl thiazolyl tetrazolium, MTT)试剂盒山羊抗小鼠IgG二抗(A0216)购自上海碧云天生物技术有限公司。

1.2 方法

1.2.1 实时定量PCR(Real-time quantitative PCR, RT-qPCR)检测circ_0015756和miR-515-5p表达 用Trizol试剂从肺癌组织、癌旁组织、BEAS-2B和A549细胞中提取总RNA,用反转录试剂盒合成circRNA、miRNA的cDNA,并利用SYBR Premix Ex Taq进行RT-qPCR。PCR扩增程序为:95 °C 10 min;95 °C 5 s,60 °C 10 s,72 °C 10 s,共40个循环。circ_0015756检测以GAPDH作为对照,miR-515-5p检测以U6作为对照。circ_0015756上游引物5'-TGG ACG GAA CCA CCT CAA TG-3',下游引物5'-CCT GAAA CCA CCC TCA CAA GT-3';GAPDH上游引物5'-GGG AAA CTG TGG CGT GAT-3',下游引物5'-GAG TGG GTG TCG CTG TTG A-3';miR-515-5p上游引物5'-CGG GTT CTC CAA AAG AAA GCA-3',下游引物5'-CAG CCA CAA AAG AGC ACA AT-3';U6上游引物5'-CTC GCT TCG GCA GCA CA-3',下游引物5'-AAC GCT TCA CGA ATT TGC GT-3'。2^{-ΔΔCt}法计算circ_0015756和miR-515-5p的相对水平。

1.2.2 细胞培养、转染 A549细胞培养在含有10%胎牛血清、1%青霉素-链霉素溶液的RPMI-1640培养液中,放入含5%二氧化碳、37 °C培养箱孵育。A549细胞融合度达到80%时进行传代。取2×10⁵个第5代A549细胞接种至6孔板,按照Lipofectamine™ 2000转染试剂说明书将si-circ_0015756、si-NC、miR-515-5p mimics、miR-NC、si-circ_0015756+anti-miR-515-5p分别转染融合度为50%的A549细胞。收集转染48 h的A549细胞,RT-qPCR检测细胞中circ_0015756或miR-515-5p的转染

效率以验证转染效果。根据转染序列不同将A549细胞分为si-NC组、si-circ_0015756组、miR-NC组、miR-515-5p组、si-circ_0015756+anti-miR-515-5p组。未转染的A549细胞记为对照(con)组。

1.2.3 MTT法检测A549活力 以2×10³个/孔的密度将转染48 h后的A549细胞接种到96孔板,常规孵育48 h后,向各孔添加20 μL的MTT溶液,孵育细胞2 h后,加入150 μL的二甲基亚砜,低速振荡15 min溶解甲瓏。用酶标仪在490 nm处测定各孔吸光度(D)值。增殖抑制率(%)=(1-实验组D值/对照组D值)×100%

1.2.4 平板克隆实验检测A549集落形成能力 将转染后的A549细胞接种6孔板,每孔3×10²个细胞,常规孵育2周,直至出现肉眼可见细胞集落。用磷酸盐缓冲液(PBS)冲洗2次,用4%多聚甲醛固定细胞集落,0.5%结晶紫染色20 min。在显微镜下对菌落进行拍照,计数大于50个细胞的集落数即为集落形成数。

1.2.5 流式细胞术检测A549凋亡率 用PBS洗涤转染A549细胞2次,收集细胞并重悬于200 μL结合缓冲液(含有10 μL Annexin V-FITC)中,室温避光孵育30 min后,添加5 μL的PI和300 μL结合缓冲液,混匀后立即上流式细胞仪分析各组样品细胞的凋亡率。

1.2.6 划痕愈合实验和Transwell实验检测A549细胞的迁移能力 (1) 划痕愈合实验。将转染后的A549细胞配制成密度为1×10³个/mL的细胞悬液,取500 μL加入6孔板中,培养过夜形成单层细胞。10 μL移液枪头在单层细胞表面划一横线,PBS冲洗3次,去除划伤脱落的细胞。在显微镜下拍照,测量初始划痕宽度。放入含5%二氧化碳、37 °C培养箱孵育24 h,测量24 h划痕宽度。迁移距离(μm)=初始划痕宽度-24 h划痕宽度。(2) Transwell实验。用无血清培养液重悬转染后的A549细胞,配制成密度为1×10⁶个/mL的细胞悬液。取200 μL加入到Transwell小室上腔,取500 μL含10%胎牛血清的培养液加入到24孔板中,37 °C培养箱孵育24 h后,取出小室,4%多聚甲醛固定穿膜细胞,0.5%结晶紫染色。显微镜下随机选择3个视野计数,以平均值表示迁移细胞数。

1.2.7 蛋白质印迹法检测A549中HMGB3蛋白表达 用RIPA缓冲液裂解转染后的A549细胞,12 000 r/min离心10 min,去除细胞碎片,BCA试剂盒分析上清液中的蛋白质含量,通过聚丙烯酰胺凝胶电泳分析后,湿转蛋白到聚偏二氟乙烯膜上。将膜在含5%脱脂

奶粉的Tris缓冲盐水-0.1% Tween 20缓冲液中室温封闭1 h, 然后与一抗(HMGB3为1:500稀释, GAPDH为1:1 500稀释)在4 °C条件下孵育过夜。将膜与对应的二抗(1:600稀释)室温孵育1 h后, 用ECL试剂盒观察印迹。采用Quantity One软件测量条带灰度。以HMGB3和GAPDH灰度值比值表示HMGB3蛋白的相对水平。

1.2.8 双荧光素酶报告实验验证 circ_0015756和 miR-515-5p、miR-515-5p和 HMGB3靶向关系 将含有 miR-515-5p结合位点的 circ_0015756或HMGB3-3'UTR序列克隆到 pmirGLO载体形成野生型(WT)荧光素酶报告质粒WT-circ_0015756、WT-HMGB3。将含有 miR-515-5p结合位点的 circ_0015756或HMGB3-3'UTR的突变序列克隆到 pmirGLO载体形成突变型(MUT)荧光素酶报告质粒MUT-circ_0015756、MUT-HMGB3。将相应的报告质粒分别与 miR-515-5p mimics或 miR-NC共转染 A549细胞, 采用荧光素酶报告基因检测试剂盒分析转染48 h后 A549细胞的萤火虫荧光素酶和海肾荧光素酶活性, 以两者的比值表示相对荧光素酶活性。

1.3 统计学方法

每组设置3个重复, 实验均独立重复3次, 数据以平均数±标准差($\bar{x}\pm s$)表示。采用SPSS 18.0软件进行统计分析, 独立样本 t 检验用于比较两组间数据差

异, 单因素方差分析和SNK- q 检验用于比较多组间数据差异。 $P<0.05$ 为差异有统计学意义。

2 结果

2.1 在肺癌组织中circ_0015756和miR-515-5p表达的检测

肺癌组织中circ_0015756的相对水平较癌旁组织显著升高($P<0.05$), 而miR-515-5p的相对水平较癌旁组织显著降低($P<0.05$)(表1)。A549细胞中circ_0015756的相对水平与BEAS-2B细胞比较显著升高($P<0.05$), miR-515-5p的相对水平与BEAS-2B细胞比较显著降低($P<0.05$)(表2)。

2.2 各组A549细胞中circ_0015756、miR-515-5p表达的检测

与con组、si-NC组比较, si-circ_0015756组A549细胞中circ_0015756的相对水平显著降低($P<0.05$), miR-515-5p的相对水平显著升高($P<0.05$); 与con组、miR-NC组比较, miR-515-5p组A549细胞中miR-515-5p的相对水平显著升高($P<0.05$); 与si-circ_0015756组比较, si-circ_0015756+anti-miR-515-5p组A549细胞中miR-515-5p的相对水平显著降低($P<0.05$)(表3)。

2.3 各组A549细胞增殖能力检测

与con组、si-NC组比较, si-circ_0015756组A549

表1 肺癌组织中circ_0015756和miR-515-5p的表达

Table 1 Expression of circ_0015756 and miR-515-5p in lung cancer

| 分组 Groups | <i>n</i> | circ_0015756 | miR-515-5p |
|------------------------------|----------|--------------|------------|
| Tissue adjacent to carcinoma | 35 | 1.01±0.11 | 1.00±0.12 |
| Lung cancer tissue | 35 | 4.60±0.33* | 0.15±0.03* |
| <i>t</i> | | 61.057 | 40.654 |
| <i>P</i> | | 0.000 | 0.000 |

* $P<0.05$, 与癌旁组织组相比。

* $P<0.05$ compared with Paracancerous tissue group.

表2 A549细胞中circ_0015756和miR-515-5p的表达

Table 2 Expression of circ_0015756 and miR-515-5p in A549 cells

| 分组 Groups | circ_0015756 | miR-515-5p |
|--|--------------|------------|
| Human normal lung mucosal epithelial cells BEAS-2B | 1.00±0.08 | 1.00±0.10 |
| Lung cancer cell A549 | 3.19±0.31* | 0.24±0.03* |
| <i>t</i> | 20.521 | 21.838 |
| <i>P</i> | 0.000 | 0.000 |

$n=9$; * $P<0.05$, 与BEAS-2B组相比。

$n=9$; * $P<0.05$ compared with BEAS-2B group.

细胞增殖抑制率显著升高 ($P < 0.05$), 集落形成数显著减少 ($P < 0.05$); 与 con 组、miR-NC 组比较, miR-515-5p 组 A549 细胞增殖抑制率显著升高 ($P < 0.05$), 集落形成数显著减少 ($P < 0.05$); 与 si-circ_0015756 组比较, si-circ_0015756+anti-miR-515-5p 组 A549 细胞增殖抑制率显著降低 ($P < 0.05$), 集落形成数显著增多 ($P < 0.05$)(表4)。

2.4 各组A549细胞凋亡率及迁移能力的检测

与 con 组、si-NC 组比较, si-circ_0015756 组 A549 细胞凋亡率显著升高 ($P < 0.05$), 迁移距离、迁移细胞数显著降低 ($P < 0.05$); 与 con 组、miR-NC 组比较, miR-515-5p 组 A549 细胞凋亡率显著升高 ($P < 0.05$), 迁移距离、迁移细胞数显著降低 ($P < 0.05$); 与 si-circ_0015756 组比较, si-circ_0015756+anti-miR-515-

5p 组 A549 细胞凋亡率显著降低 ($P < 0.05$), 迁移距离、迁移细胞数显著升高 ($P < 0.05$)(图1和表5)。

2.5 各组A549细胞中HMGB3蛋白表达的检测

与 con 组、si-NC 组比较, si-circ_0015756 组 A549 细胞 HMGB3 蛋白的相对水平显著减少 ($P < 0.05$); 与 con 组、miR-NC 组比较, miR-515-5p 组 A549 细胞 HMGB3 蛋白的相对水平显著减少 ($P < 0.05$); 与 si-circ_0015756 组比较, si-circ_0015756+anti-miR-515-5p 组 A549 细胞 HMGB3 蛋白的相对水平显著增加 ($P < 0.05$)(图2和表6)。

2.6 circ_0015756、miR-515-5p和HMGB3靶向关系的验证

Circular RNA Interactome 在线分析显示, circ_0015756 与 miR-515-5p 序列间存在特异性结合位

表3 A549细胞中circ_0015756、miR-515-5p的表达
Table 3 Expression of circ_0015756 and miR-515-5p in A549 cells

| 分组 Groups | circ_0015756 | miR-515-5p |
|---------------------------------|-------------------------|-----------------------------|
| con | 1.00±0.00 | 1.00±0.00 |
| si-NC | 1.01±0.02 | 0.99±0.04 |
| si-circ_0015756 | 0.23±0.02* [#] | 4.13±0.09* [#] |
| miR-NC | - | 0.98±0.03 |
| miR-515-5p | - | 5.39±0.10* ^{&} |
| si-circ_0015756+anti-miR-515-5p | - | 1.22±0.05 [@] |
| F | 6 757.874 | 8 981.556 |
| P | 0.000 | 0.000 |

$\bar{x} \pm s$; $n=9$; * $P < 0.05$, 与 con 组相比; [#] $P < 0.05$, 与 si-NC 组相比; [&] $P < 0.05$, 与 miR-NC 组相比; [@] $P < 0.05$, 与 si-circ_0015756 组相比。

-: circ_0015756 表达变化影响 miR-515-5p 的表达, 但 miR-515-5p 表达变化对 circ_0015756 无影响。

$\bar{x} \pm s$; $n=9$; * $P < 0.05$ compared with con group; [#] $P < 0.05$ compared with si-NC group; [&] $P < 0.05$ compared with miR-NC group; [@] $P < 0.05$ compared with si-circ_0015756 group. -: the expression change of circ_0015756 affects the expression of miR-515-5p, but the expression change of miR-515-5p has no effect on circ_0015756.

表4 A549细胞抑制率和集落形成数的检测
Table 4 Detection of inhibition rate and colony forming number of A549 cells

| 分组 Groups | 抑制率/% Inhibition rate /% | 集落形成数/个 Colony forming number /piece |
|---------------------------------|------------------------------|---|
| con | 0.00±0.00 | 128.22±3.79 |
| si-NC | 0.01±0.01 | 127.33±4.24 |
| si-circ_0015756 | 54.71±1.81* [#] | 65.67±2.31* [#] |
| miR-NC | 0.02±0.01 | 127.89±4.12 |
| miR-515-5p | 64.33±2.28* ^{&} | 53.56±1.95* ^{&} |
| si-circ_0015756+anti-miR-515-5p | 15.81±0.82 [@] | 113.11±3.38 [@] |
| F | 5 135.331 | 894.356 |
| P | 0.000 | 0.000 |

$\bar{x} \pm s$; $n=9$; * $P < 0.05$, 与 con 组相比; [#] $P < 0.05$, 与 si-NC 组相比; [&] $P < 0.05$, 与 miR-NC 组相比; [@] $P < 0.05$, 与 si-circ_0015756 组相比。

$\bar{x} \pm s$; $n=9$; * $P < 0.05$ compared with con group; [#] $P < 0.05$ compared with si-NC group; [&] $P < 0.05$ compared with miR-NC group;

[@] $P < 0.05$ compared with si-circ_0015756 group.

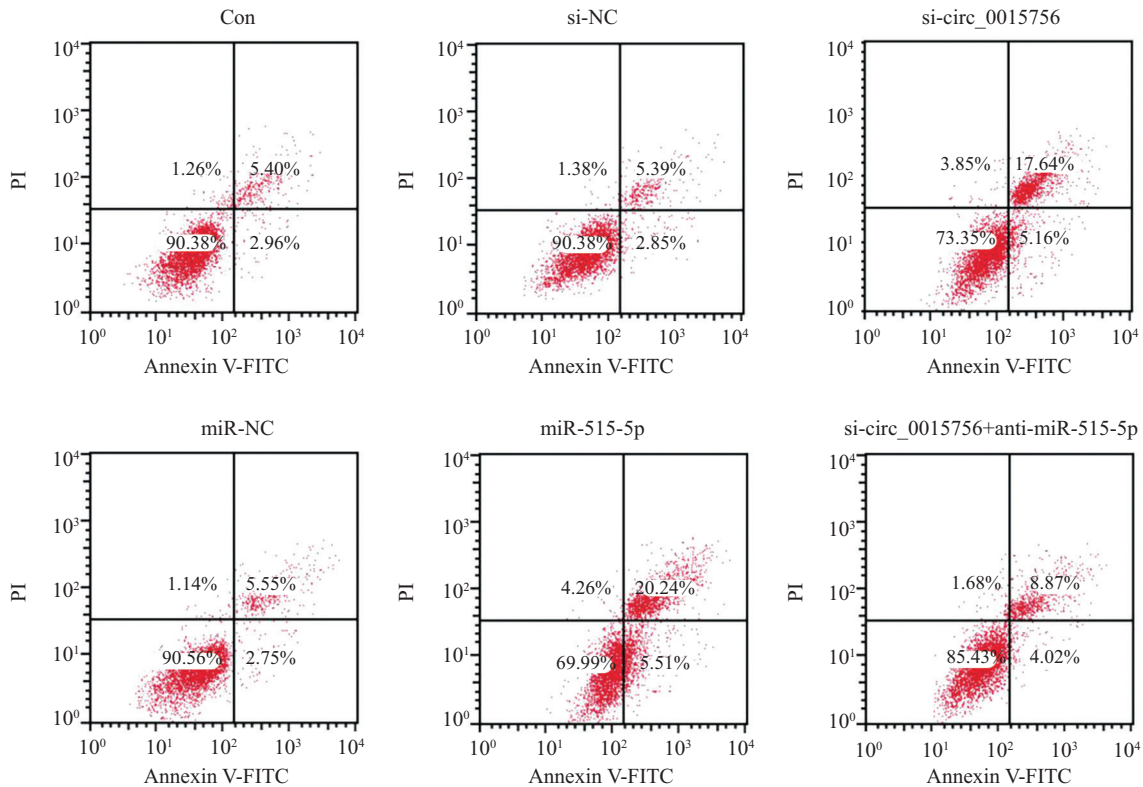


图1 A549细胞凋亡率的检测

Fig.1 Detection of apoptosis rate of A549 cells

表5 A549细胞凋亡率及迁移距离的检测

Table 5 Detection of apoptosis rate and migration distance of A549 cells

| 分组 Groups | 凋亡率/% Apoptosis rate /% | 迁移距离/ μm Migration distance / μm | 迁移细胞数/个 Migratory cell number /piece |
|---------------------------------|----------------------------|---|---|
| con | 8.36±0.42 | 172.48±7.57 | 157.67±3.83 |
| si-NC | 8.24±0.49 | 175.43±7.73 | 159.56±4.79 |
| si-circ_0015756 | 22.80±0.80*# | 91.36±4.14*# | 75.78±1.47*# |
| miR-NC | 8.30±0.48 | 174.15±9.10 | 160.44±4.90 |
| miR-515-5p | 25.75±0.89*# | 76.86±2.94*# | 63.33±1.49*# |
| si-circ_0015756+anti-miR-515-5p | 12.89±0.63@ | 147.29±4.43@ | 125.22±4.05@ |
| F | 1 366.627 | 435.086 | 1 275.570 |
| P | 0.000 | 0.000 | 0.000 |

$\bar{x}\pm s$; n=9; *P<0.05, 与con组相比; #P<0.05, 与si-NC组相比; &P<0.05, 与miR-NC组相比; @P<0.05, 与si-circ_0015756组相比。

$\bar{x}\pm s$; n=9; *P<0.05 compared with con group; #P<0.05 compared with si-NC group; &P<0.05 compared with miR-NC group; @P<0.05 compared with si-circ_0015756 group.

点(图3A)。TargetsCan在线分析显示, miR-515-5p与HMGB3-3'UTR存在特异互补序列(图3B)。同与WT-circ_0015756或WT-HMGB3共转染, 转染miR-515-5p mimics后A549细胞的相对荧光素酶活性与转染miR-NC后A549细胞的相对荧光素酶活性比较显著下降(P<0.05); 同与MUT-circ_0015756或MUT-HMGB3共转染, 转染miR-515-5p mimics后A549细胞的相对荧光素

酶活性与转染miR-NC比较差异无统计学意义(表7)。

3 讨论

circ_0015756在癌症进展的研究中已被证实具有致癌作用, 研究发现, 卵巢癌中circ_0015756水平升高, 干扰circ_0015756可降低卵巢癌细胞的体外增殖、迁移和侵袭能力, 抑制体内肿瘤生长^[8]。干

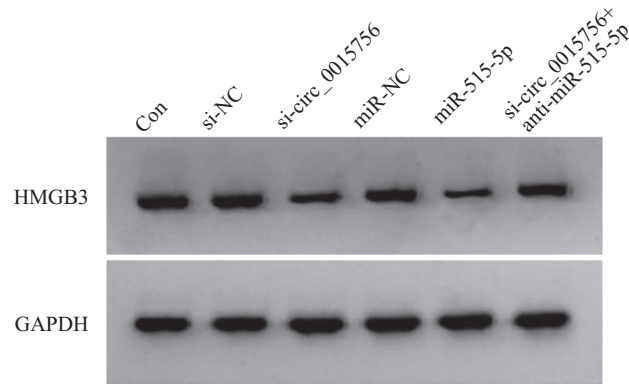


图2 HMGB3蛋白表达的检测

Fig.2 Detection of HMGB3 protein expression

表6 A549细胞中HMGB3蛋白表达的检测

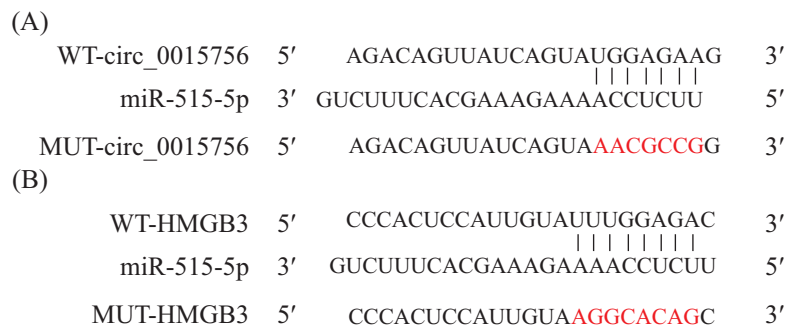
Table 6 Detection of HMGB3 protein expression in A549 cells

| 分组 Groups | HMGB3 |
|---------------------------------|-----------------------------|
| con | 0.76±0.04 |
| si-NC | 0.75±0.04 |
| si-circ_0015756 | 0.33±0.02* [#] |
| miR-NC | 0.77±0.04 |
| miR-515-5p | 0.23±0.02* ^{&} |
| si-circ_0015756+anti-miR-515-5p | 0.62±0.04 [@] |
| <i>F</i> | 425.900 |
| <i>P</i> | 0.000 |

$\bar{x} \pm s$; $n=9$; * $P<0.05$, 与con组相比; [#] $P<0.05$, 与si-NC组相比; [&] $P<0.05$, 与miR-NC组相比; [@] $P<0.05$, 与si-circ_0015756组相比。

$\bar{x} \pm s$; $n=9$; * $P<0.05$ compared with con group; [#] $P<0.05$ compared with si-NC group; [&] $P<0.05$ compared with miR-NC group;

[@] $P<0.05$ compared with si-circ_0015756 group.



A: circ_0015756和miR-515-5p的互补序列; B: miR-515-5p和HMGB3的互补序列。标红处为突变位点。

A: circ_0015756 and miR-515-5p complementary sequences; B: miR-515-5p and HMGB3 complementary sequence. The place marked in red is the mutation site.

图3 circ_0015756、miR-515-5p的靶向序列预测及miR-515-5p、HMGB3的靶向序列预测

Fig.3 circ_0015756, miR-515-5p targeting sequence prediction and miR-515-5p, HMGB3 targeting sequence prediction

扰 circ_0015756通过靶向 miR-610上调成纤维细胞生长因子受体1(fibroblast growth factor receptor 1, FGFR1)蛋白表达诱导肝癌细胞凋亡和周期阻滞^[9]。本研究中肺癌组织中 circ_0015756的相对

水平显著升高, 而干扰 circ_0015756显著降低肺癌 A549细胞的增殖、迁移能力, 诱导细胞凋亡, 这与 circ_0015756在其他癌症中的作用一致^[8-9]。miR-515-5p在包括肺癌、乳腺癌和膀胱癌在内的多种

表7 双荧光素酶报告实验

Table 7 Double luciferase report experiment

| 分组 Groups | WT-circ_0015756 | MUT-circ_0015756 | WT-HMGB3 | MUT-HMGB3 |
|--------------|-----------------|------------------|------------|-----------|
| miR-NC | 0.99±0.03 | 0.96±0.05 | 0.98±0.08 | 0.98±0.05 |
| miR-515-5p | 0.35±0.02* | 0.97±0.07 | 0.21±0.02* | 0.96±0.06 |
| <i>t</i> | 53.251 | 0.349 | 28.013 | 0.768 |
| <i>P</i> | 0.000 | 0.732 | 0.000 | 0.454 |

$\bar{x}\pm s$; $n=9$; * $P<0.05$, 与miR-NC组相比。

$\bar{x}\pm s$; $n=9$; * $P<0.05$ compared with miR-NC group.

癌中发挥了抑癌作用^[10-12]。研究显示, miR-515-5p通过靶向白细胞介素6(interleukin 6, IL-6)抑制肝癌细胞迁移和侵袭来抑制肝癌转移,从而阻断肝癌进展^[13]。在前列腺癌中miR-515-5p介导敲减circ_0057553对癌细胞糖酵解、增殖、迁移和侵袭的抑制作用^[14]。本研究发现肺癌组织中miR-515-5p的相对水平显著降低,过表达miR-515-5p明显诱导A549细胞凋亡,抑制细胞增殖和迁移。同时,本研究发现miR-515-5p为circ_0015756的直接靶点,且在A549细胞中miR-515-5p的表达受到circ_0057553的负调控。干扰circ_0015756与过表达miR-515-5p的抗肿瘤效果类似,提示circ_0015756可能靶向miR-515-5p调控肺癌进展。进一步研究表明,抑制circ_0015756表达明显减弱干扰circ_0015756表达对A549细胞增殖、迁移抑制和凋亡促进作用,这表明肺癌中存在circ_0015756/miR-515-5p调控途径,circ_0015756靶向下调miR-515-5p参与肺癌进展。

miRNA通过与mRNA的3'UTR结合而影响mRNA表达是其参与肿瘤进展的重要机制,研究显示,miR-515-5p通过靶向磷脂酰肌醇3-激酶催化亚基 α (phosphatidylinositol 3-kinase catalyzed subunit α -polypeptide, PIK3CA)抑制鼻咽癌细胞增殖和放疗耐受^[15]。miR-515-5p的明显下调性别决定相关基因9(sex-determining region Y box 9, SOX9)表达水平,并增强胃癌细胞对奥沙利铂的耐药性^[16]。本研究发现HMGB3是miR-515-5p的潜在靶基因。HMGB3已被证实为肺癌的致癌基因,研究报告,HMGB3作为miR-513b的靶点可促进肺癌细胞增殖和转移^[17]。长链非编码RNA(lncRNA) HOXA末端转录本反义RNA(HOXA transcript at the distal tip, HOTTIP)通过调控miR-615-3p/HMGB3轴促进缺氧诱导的肺癌细胞糖酵解^[18]。此外,HMGB3还可作为circ_0001313/miR-452和circ_102179/miR-330-5p分子轴的下游mRNA,参

与其对肺癌细胞的促增殖、促迁移作用^[19-20]。本研究证实HMGB3是miR-515-5p的直接靶点,过表达miR-515-5p或干扰circ_0015756均可下调HMGB3蛋白表达水平,且抑制miR-515-5p表达明显减弱干扰circ_0015756表达对HMGB3蛋白表达的抑制作用。这表明circ_0015756通过调控miR-515-5p/HMGB3轴参与肺癌进展。

总之,本研究首次阐明了circ_0015756在肺癌中的表达模式和作用,证实了干扰circ_0015756通过靶向上调miR-515-5p/HMGB3轴可抑制肺癌细胞增殖和迁移,诱导细胞凋亡,进而抑制肺癌进展。这些发现为肺癌治疗提供潜在有效靶点。

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