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miR–219–5p对非小细胞肺癌细胞增殖、凋亡与侵袭的影响及其机制

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[摘　要]　**目的：**探索miR-219-5p对非小细胞肺癌(non-small cell lung cancer，NSCLC)细胞增殖、凋亡及侵袭的影响，并探讨其机制。**方法：**采用RT-PCR检测miR-219-5p在NSCLC细胞系H1299，A549，H1975及正常肺上皮细胞系BEAS-2B中的表达。将NSCLC细胞系H1299分成对照组和miR-219-5p组，用Lipofectamine 2000分别转染miR-219-5p scramble和miR-219-5p mimics，采用MTT法、流式细胞术及Transwell实验分别检测比较两组细胞增殖、凋亡及侵袭能力，Western印迹测定表皮生长因子受体(epidermal growth factor receptor，EGFR)及裂解型多聚腺苷二磷酸核糖聚合酶(poly ADP ribose polymerase，PARP)在两组细胞中的表达。**结果：**miR-219-5p在H1299，A549和H1975细胞系中的表达量均低于BEAS-2B，差异有统计学意义(P<0.05)；MTT实验显示在48，72，96及120 h，miR-219-5p组$OD_{490\ nm}$值显著低于对照组，差异有统计学意义(P<0.05)；miR-219-5p组细胞凋亡率显著高于对照组(13.33%±1.20% vs 3.43%±0.12%)，差异有统计学意义(P<0.01)；miR-219-5p组侵袭细胞数显著少于对照组(67.5±9.9 vs 189.5±16.7)，差异有统计学意义(P<0.05)；miR-219-5p组EGFR蛋白相对表达量为0.35±0.07，miR-219-5p组EGFR蛋白相对表达量显著低于对照组(1.0)，差异有统计学意义(P<0.01)；miR-219-5p组裂解型PARP蛋白相对表达量显著高于对照组(2.74±0.17 vs 1.0)，差异有统计学意义(P<0.01)。**结论：**miR-219-5p可抑制NSCLC的细胞增殖和侵袭并促进其凋亡，其机制可能与下调EGFR及上调PARP的表达有关。

[关键词]　miR-219-5p；非小细胞肺癌；增殖；侵袭；凋亡

Effect of miR-219-5p on proliferation, apoptosis and invasion of non-small cell lung cancer and its mechanism

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Abstract　**Objective:** To explore the role of miR-219-5p in the proliferation, apoptosis and invasion of non-small cell lung cancer (NSCLC) and study its mechanism. **Methods:** The expression of miR-219-5p in NSCLC cell lines H1299, A549, H1975 and BEAS-2B were evaluated, real-time quantitative PCR (RT-PCR) was determined. H1299 cell line was divided into a control group and a miR-219-5p group, which was transfected with miR-219-5p scramble and miR-219-5p mimic by Lipofectamine 2000, respectively. The proliferation, apoptosis and invasive of the two groups

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was measured by MTT assay, flow cytometry and Transwell assay. The expression level of EGFR and cleaved poly ADP ribose polymerase (PARP) protein was measured by Western blot. **Results:** The expression level of miR-219-5p in H1299, A549 and H1975 cell line was significantly lower than that in BEAS-2B (P<0.05). MTT showed that the value of $OD_{490\,nm}$ of the miR-219-5p group was significantly lower than that in the control group in 48, 72, 96 and 120 h (P<0.05). The apoptosis rate of miR-219-5p was significantly higher than that in the control group (13.33%±1.20% vs 3.43%±0.12%; P<0.01). The number of invasive cells in the miR-219-5P group was significantly less than that in the control group (67.5±9.9 vs 189.5±16.7; P<0.01). The expression level of EGFR protein of miR-219-5p group was significantly lower than that in the control group (0.35±0.07 vs 1.0; P<0.01). The expression level of PARP protein of miR-219-5p group was significantly higher than that in the control group, (2.74±0.17 vs 1.0; P<0.01). **Conclusion:** miR-219-5p could restrain cell growth and invasion, and induce apoptosis in NSCLC cells, which may be possibly related with upregulated EGFR and downregulated PARP.

Keywords miR-219-5p; non-small cell lung cancer; proliferation; invasion; apoptosis

肺癌是我国最常见的恶性肿瘤之一[1]。据统计，2010年我国肺癌新发病例60.59万以上，死亡病例约48.66万，肺癌已成为肿瘤致死的首要原因[1]。非小细胞肺癌(non-small cell lung cancer，NSCLC)约占肺癌的80%[2]。目前，手术仍然是早期NSCLC的主要治疗手段，但30%~70%的患者发生术后复发、转移，且对于多数患者来说，发现时往往已是晚期[3]。miRNA是一类能控制基因转录后水平和翻译水平的非编码小RNA，长度约19~25个核苷酸[4]。它们对维持细胞正常功能，控制细胞生长、发育、分化等过程起关键作用[5]。miRNAs与多种人类疾病的发生有着密切关系，研究证实：miRNAs在肿瘤的发生和发展中起重要作用，如慢性淋巴细胞白血病[6]、乳腺癌[7]、肺癌[8]等。miRNAs是肿瘤诊断及预后的重要标志。miR-219-5p被发现在多种肿瘤中表达下调且参与调控肿瘤发生发展，如胃癌[9]、髓母细胞瘤[10]、肝细胞癌[11]、结直肠癌[11]等。但是，miR-219-5p在NSCLC中的功能尚不明确。本研究旨在研究miR-219-5p对NSCLC增殖、凋亡和侵袭的影响，并对其机制作初步探索。

1 材料与方法

1.1 材料

人NSCLC细胞系H1299，A549，H1975及正常肺上皮细胞系BEAS-2B均购自美国ATCC细胞库，细胞培养37 ℃孵箱购自美国Thermo公司，Western印迹仪购自美国Bio-Rad公司，流式细胞仪购自美国BD公司，Dye670染料购自美国BD公司；Annexin V/PI凋亡检测试剂盒购自天津三箭生物技术有限公司；DMEM培养基、胎牛血清、胰蛋白酶均购自美国Hyclone公司，TRIzol购自美国Invitrogen公司；实验所需一抗(抗EGFR，抗PARP)购自美国Cell Signal Technology公司；抗兔辣根过氧化物酶偶联二抗购自武汉博士德生物科技有限公司；miR-219-5p mimics及scramble均由广州锐博公司合成。

1.2 细胞培养分组及转染

将NSCLC细胞系H1299，A549，H1975及正常肺上皮细胞系BEAS-2B加入DMEM培养基，于37 ℃、5% CO_2的条件培养于培养箱中，48 h后消化传代。将NSCLC细胞系H1299分成对照组和miR-219-5p组，对照组和miR-219-5p组采用Lipofectamine 2000 reagent (Invitrogen，USA)分别转染miR-219-5p scramble和miR-219-5p mimics，未转染对照组用PBS处理，作为空白对照。miR-219-5p mimics转染序列：miR-219-5p mimics-sense 5'-AAAAGAATTCCCACTT-CCCACTCCAGACATT-3'，antisense 5'-AAAG-CGGCCGCCCCTCACTTCTCCGTAACCC-3'；对照组转染序列：miR-219-5p scramble-sense 5'-UUCUCCGAACGUGUCACGUTT-3'，miR-219-5p scramble-antisense 5'-ACGUGACACG-UUCGGAGAATT-3'。

1.3 RNA 提取及实时定量 PCR

用All-in-One microRNA抽提试剂盒和All-in-One miRNA qRT-PCR检测试剂盒提取和分离miRNAs，ABI Prism 7700 system的SYBR Green Reagents(日本TaKaRa公司)进行qRT-PCR，在ABI

7500实时定量PCR仪中，以U6小核RNA作为内参，使用$2^{-\Delta\Delta Ct}$方法定量，量化miR-219-5p的相对表达水平。

1.4 细胞增殖实验

采用MTT法，将两组细胞消化成单细胞悬液，在96孔板上以1×10^{3}个/孔接种，每孔培养基体积200 μL。在分别培养0，24，48，72，96 h后，每孔加入20 μL 5 mg/mL的MTT溶液，孵育4 h后每孔加入200 μL DMSO，摇床上充分震荡。用酶标仪在490 nm波长测定各孔吸光度，以时间为横坐标，吸光度值为纵坐标绘制细胞增殖曲线。

1.5 细胞凋亡实验

采用流式细胞术，用Annexin V/PI染色检测，将两组细胞消化成单细胞悬液后，PBS清洗2次，并使用Binding Buffer重悬，加入相应比例的Annexin V抗体，避光染色10 min后加入适量PBS溶液以及PI染料，流式细胞仪检测Annexin V阳性细胞比例来确定细胞凋亡的变化。

1.6 Western 印迹

采用Western印迹法，将两组细胞用RIPA细胞裂解液冰上裂解30 min后，变性、上样，以每孔30 μg总蛋白上样，浓缩胶80 V电泳40 min，分离胶100 V电泳2 h。常规湿法转膜，加入EGFR，PARP一抗，浓度为1:200，37 ℃孵育4 h，二抗(1:500)孵育过夜，ECL液显影，Quantity One 1-D分析软件对蛋白质印迹条带进行定量。目的蛋白相对表达量=目的蛋白测定值/GAPDH，实验重复3次，取平均值。

1.7 细胞侵袭能力测定

采用Transwell实验，两组各取2×10^{4}个细胞，种在Transwell小室的碳酸磷脂表面，上室BioCoat TM包被Matrigel基质胶(美国BD Biosciences公司)，37 ℃培养24 h。取出上层小室，将膜下面的细胞与1%多聚甲醛混合，用0.2%结晶紫溶液染色15 min。用显微镜计数进入膜下的细胞量，随机取10个视野，计算平均值。实验重复3次，取平均值。

1.8 统计学处理

用SPSS 17.0统计软件分析。计量资料用均数±标准差($\bar{x}\pm s$)表示，组间比较采用t检验，以P<0.05为差异有统计学意义。

2 结果

2.1 miR-219-5p 低表达于 NSCLC 细胞系

qRT-PCR结果示：正常肺上皮细胞系BEAS-2B miR-219-5p相对表达量为1.0，在NSCLC细胞系H1299，A549，H1975中miR-219-5p相对表达量分别为0.29±0.032，0.35±0.029和0.39±0.043，均低于BEAS-2B，差异有统计学意义(P<0.01，图1)。

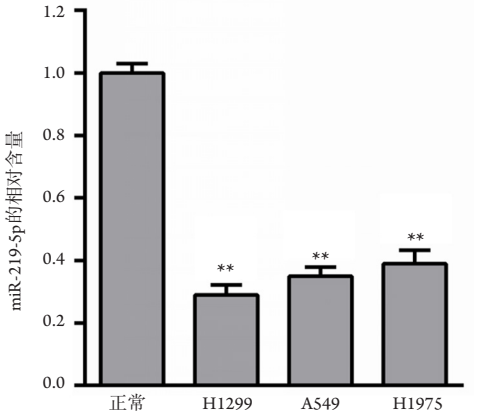

图1 miR-219-5p在正常肺上皮细胞系和非小细胞肺癌细胞系中的表达

Figure 1 Expression level of miR-219-5p in normal lung cell line and NSCLC cell line

与正常细胞比较，**P<0.01。

Compared with the normal cells, **P<0.01.

2.2 miR-219-5p 过表达抑制 NSCLC 细胞增殖

H1299肺癌细胞系转染后，qRT-PCR检测两组miR-219-5p的表达量示：miR-219-5p组miR-219-5p相对表达量为11.63±0.58，对照组为1.0，差异有统计学意义(P<0.01，图2A)，提示转染效果可，可行后续实验。

MTT实验示：0，24及48 h，miR-219-5p组与对照组的$OD_{490\ nm}$值分别为0.14±0.02 vs 0.13±0.04，0.23±0.05 vs 0.22±0.04，0.43±0.07 vs 0.56±0.06，差异均无统计学意义(P>0.05)；72，96，120 h，miR-219-5p组与对照组的$OD_{490\ nm}$值分别为0.76±0.09 vs 1.39±0.14，1.29±0.17 vs 2.37±0.25，2.12±0.23 vs 3.63±0.34，差异均有统计学意义(P<0.001，图2B)。

2.3 miR-219-5p 过表达促进 NSCLC 细胞凋亡

流式细胞术示：NC组细胞凋亡率为3.43%±0.12%，miR-219-5p组为13.33%±1.20%，

miR-219-5p组细胞凋亡率显著高于NC组，差异有统计学意义(P<0.01，图3)。

2.4 miR-219-5p 过表达抑制 NSCLC 细胞侵袭

200倍视野下，miR-219-5p组侵袭细胞数为67.5±9.9，对照组为189.5±16.7，miR-219-5p组侵袭细胞数显著少于对照组，差异有统计学意义(P<0.01，图4)。

2.5 miR-219-5p 过表达下调 EGFR 且上调 PARP 的表达

Western印迹示：miR-219-5p组EGFR蛋白相对表达量为0.35±0.07，显著低于对照组的1.0，差异有统计学意义(P<0.01，图5A)。

miR-219-5p组裂解型PARP蛋白相对表达量为2.74±0.17，显著高于对照组的1.0，差异有统计学意义(P<0.01，图5B)。

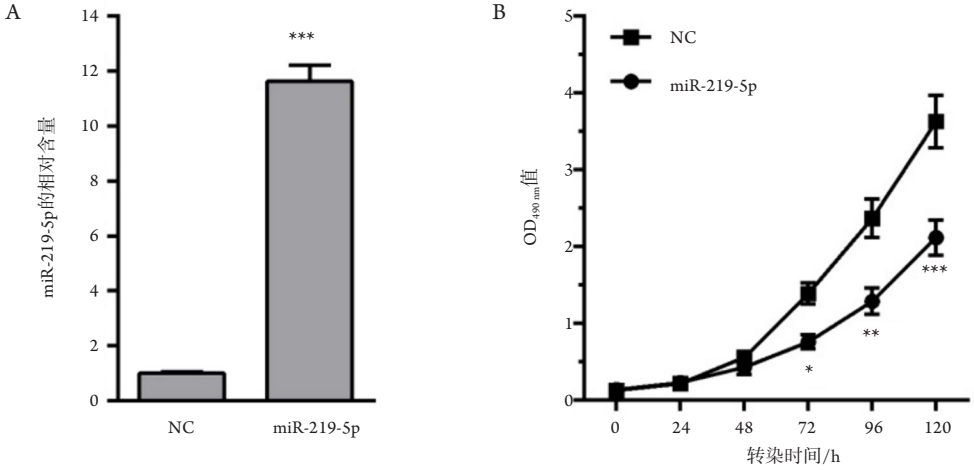

图2 miR-219-5p过表达抑制非小细胞肺癌细胞增殖

Figure 2 Overexpression of miR-219-5p inhibit the proliferation of NSCLC cell line

(A)两组miR-219-5p相对表达量的比较；(B)两组细胞增殖曲线。与对照组比较，*P<0.05，**P<0.01，***P<0.001。

(A) Expression level of miR-219-5p in the two groups; (B) Proliferation curve of the two groups. Compared with the control group, *P<0.05, **P<0.01, ***P<0.001.

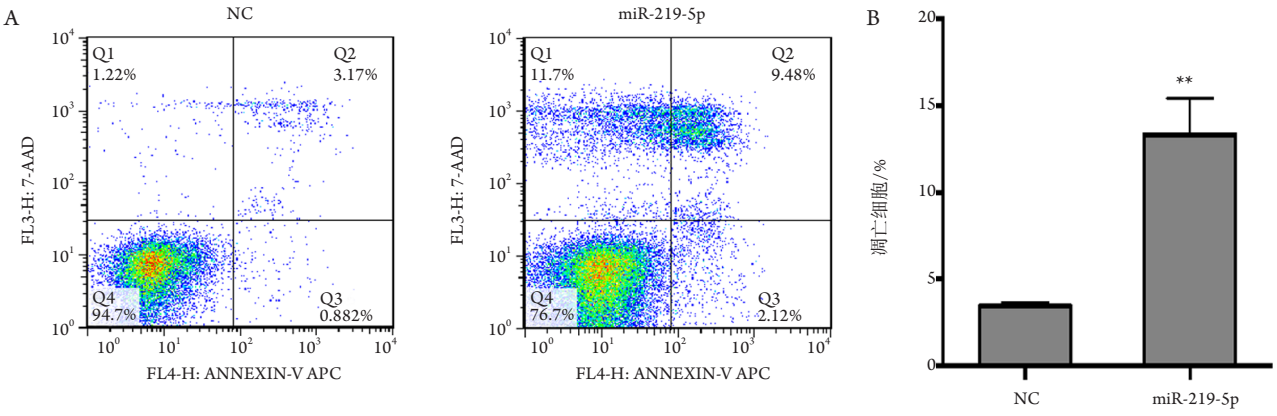

图3 miR-219-5p过表达促进非小细胞肺癌细胞凋亡

Figure 3 Overexpression of miR-219-5p promote the apoptosis of NSCLC cell line

(A)流式细胞术示两组凋亡情况；(B)两组凋亡率比较。与对照组比较，**P<0.01。

(A) Apoptosis ability of the two groups was shown by flow cytometry; (B) Apoptosis rate was compared between the two groups. Compared with the control group, **P<0.01.

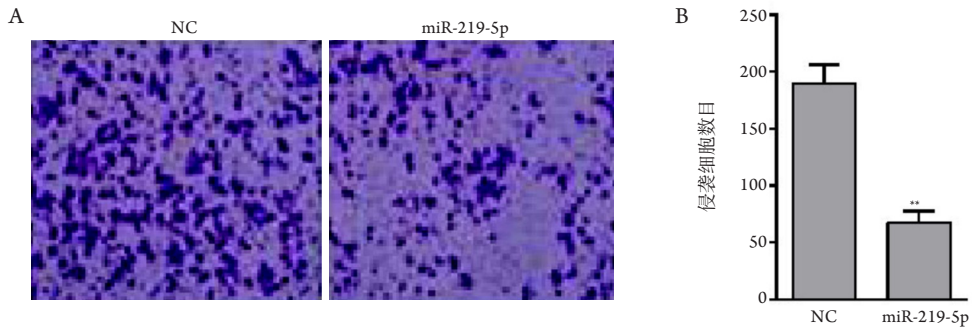

图4 miR-219-5p过表达抑制非小细胞肺癌细胞侵袭

Figure 4 Overexpression of miR-219-5p inhibits the invasion of NSCLC cell line

(A)两组Transwell实验；(B)两组侵袭细胞数比较。与对照组比较，**P<0.01。

(A) Transwell assay of the two groups; (B) Comparison of the number of invasive cells between the two groups. Compared with the control group, **P<0.01.

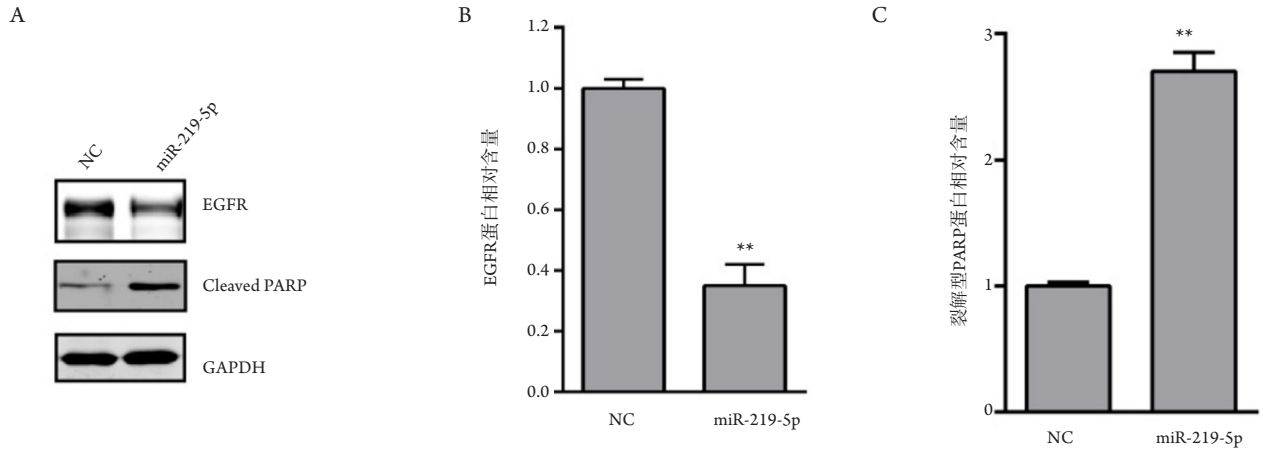

图5 miR-219-5p过表达下调EGFR和上调PARP的表达

Figure 5 Overexpression of miR-219-5p downregulates the expression of EGFR and upregulate the expression of PARP

(A)蛋白印迹示两组EGFR和裂解型PARP蛋白的表达；(B)两组EGFR蛋白表达的比较；C)两组裂解型PARP蛋白表达的比较。与对照组比较，**P<0.01。

(A) Expression level of EGFR and cleaved PARP in the two groups was measured by Western blot; (B) Expression level of EGFR protein in the two groups; (C) Expression level of cleaved PARP protein in the two groups. Compared with the control group, **P<0.01.

3 讨论

NSCLC是常见的肺癌类型，5年生存率低于20%[12]。研究[13]表明：miRNAs通过靶向调控基因表达，对肿瘤的发生发展等过程起着重要的调控作用。如：miR-34a通过直接抑制CD44的表达，抑制前列腺癌干细胞的生长和转移[14]。miR-205过表达诱导肾癌细胞G_0/G_1周期阻滞和凋亡，参与抑制细胞增殖、克隆形成、侵袭和转移[15]。过表达miR-155和低表达的let-7a均与肺癌的不良预后有关[8]。在结肠癌中，miR-135b在散发性和炎症性肠病相关性大肠癌中表达上调，与肿瘤分期及临床预后差有关[16]。

miR-219-5p是一个与肿瘤高度相关的miRNAs家族成员，靶向glypican-3抑制肝癌细胞增殖[11]，靶向calcyphosin抑制结直肠癌生长和侵袭[11]。本研究发现miR-219-5p在NSCLC细胞系中的表达水平低于正常肺上皮细胞系，提示miR-219-5p可能作为肿瘤抑制因子与NSCLC的发生、发展、转移有关。建立稳定过表达miR-219-5p组，通过MTT及Transwell小室实验发现miR-219-5p过表达可显著降低H1299细胞增殖和侵袭能力。

miR-219-5p通过靶向EGFR 3'-UTR抑制MAPK及P13K受体型酪氨酸激酶信号通路，从而阻碍胶母细胞瘤细胞生长、增殖和迁移[10]。EGFR作为一种跨膜酪氨酸激酶，通过绑定表皮生长因子(epidermal growth factor，EGF)、TGF-α、双调蛋白(amphiregulin，AREG)、肝素结合性表皮生长因子(heparin-binding epidermal growth factor，HBEGF)、β细胞素(betacellulin，BTC)、表皮调节素或epigen等配体激活细胞内或下游信号转导途径[17]。研究[18]发现：EGFR是多个miRNA的靶基因，如miR-133a通过靶向EGFR调控EGFR/Akt信号通路，参与调节乳腺癌细胞周期和增殖。miR-302b靶向EGFR抑制人肝癌SMMC-7721细胞增殖[19]，miR-133b靶向EGFR抑制NSCLC生长[20]。本研究发现：miR-219-5p过表达抑制EGFR的蛋白表达水平，意味着不仅是胶质母细胞瘤，在NSCLC中EGFR也是miR-219-5p的靶基因，综合前人研究[9-11]，得出miR-219-5p具有多个靶基因，在不同肿瘤中可通过靶向不同的基因调控肿瘤发生发展。

EGFR通常激活细胞质MAPK-ERK信号通路，ERK的激活常被用作EGFR抑制剂作用的生物标志物[21]。MAPK-ERK级联途径是调节正常细胞增殖、存活及分化的关键信号通路，MAPK级联的异常与肿瘤等人类疾病有重要关联[22]。另外，EGFR还可激活PI3K-AKT，JAK-STAT，蛋白激酶C和磷脂酶D等信号通路，这些信号途径被EGFR激活将导致肿瘤细胞增殖、血管生成、侵袭、转移，抑制细胞凋亡等生物过程[23]。据研究[19]报道：基因扩增、突变、配体过量表达等多种机制都可导致EGFR信号途径的激活。本研究中，miR-219-5p过表达时，EGFR表达明显降低，意味着EGFR介导的信号途径受抑制，miR-219-5p可能发挥与之在胶母细胞瘤中类似的作用机制，即通过抑制MAPK，PI3K等信号通路的传导，减弱NSCLC细胞的生长和侵袭。

PARP为113 kD蛋白，在细胞凋亡过程中，PARP裂解为89 kD和24 kD的两个小片段[24]。与对照组相比，PARP在miR-219-5p过表达时裂解明显增多，表明miR-219-5p过表达诱导NSCLC细胞凋亡。研究发现：EGFR介导的信号转导途径参与诱导细胞凋亡发生过程。如没食子儿茶素-3-没食子酸酯(epigallocatechin-3-gallate，EGCG)通过抑制EGFR/ERK途径及cyclin B1/CDK1复合体抑制人甲状腺未分化癌细胞的生长、诱导细胞凋亡[25]。厚朴酚通过抑制EGFR和PI3K/Akt信号通路诱导人前列腺癌细胞的凋亡作用[26]。本研究中miR-219-5p过表达可能通过抑制EGFR及其信号通路，诱导细胞凋亡信号途径，使PARP蛋白加速裂解，促进细胞凋亡，有助于抑制NSCLC增殖和侵袭过程。

miRNA通常与靶基因的3'-UTR相结合，对靶基因进行转录后调控[27]，之前的研究[10]证实miR-219-5p在胶质母细胞瘤中作用于EGFR 3'-UTR，因此，我们推测miR-219-5p在NSCLC中也很可能是通过结合EGFR 3'-UTR，从而对EGFR的转录后水平进行调控。目前，已有两种针对抑制EGFR信号途径的策略被成功开发，一种是单克隆抗体靶向EGFR胞外区，一种是小分子酪氨酸激酶抑制剂(tyrosine kinase inhibitor，TKI)作用于胞内酪氨酸激酶结构域，通过阻碍配体–受体互相作用，达到抑制EGFR信号途径的作用[21]。基于EGFR小分子TKI开发出的靶向药物如易瑞沙、吉非替尼及厄洛替尼的研发对部分NSCLC患者有一定的治疗效果[28]。因此，本研究中miR-219-5p过表达对EGFR信号途径的抑制对开发新的肿瘤治疗策略具有重要意义。

当然，本研究也存在一些不足，如miR-219-5p在NSCLC组织及癌旁组织中的表达情况如何，与临床病理因素的关系如何等，值得进一步研究。

综上所述，本研究发现miR-219-5p能通过下调EGFR和上调PARP蛋白表达，抑制NSCLC的增殖和侵袭，促进凋亡。miR-219-5p有望成为NSCLC潜在的诊断和预后标志物，并可能成为治疗NSCLC的新靶标。

参考文献

1. Cui W, Zhang S, Shan C, et al. microRNA-133a regulates the cell cycle and proliferation of breast cancer cells by targeting epidermal growth factor receptor through the EGFR/Akt signaling pathway[J]. FEBS J, 2013, 280(16): 3962-3974.
2. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial[J]. Lancet, 2016, 387(10027): 1540-1550.
3. Spigel DR, Reckamp KL, Rizvi NA, et al. A phase III study (CheckMate 017) of nivolumab (NIVO; anti-programmed death-1 [PD-1]) vs docetaxel (DOC) in previously treated advanced or metastatic squamous (SQ) cell non-small cell lung cancer (NSCLC)[J]. J Clin Oncol, 2015, 33 (Suppl): abstr 8009.
4. Feng X, Wang Z, Fillmore R, et al. MiR-200, a new star miRNA in human cancer[J]. Cancer Lett, 2014, 344(2): 166-173.

5. Huang Y, Shen XJ, Zou Q, et al. Biological functions of microRNAs: a review[J]. J Physiol Biochem, 2011, 67(1): 129-139.
6. Calin GA, Ferracin M, Cimmino A, et al. A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia[J]. N Engl J Med, 2005, 353(17): 1793-1801.
7. Iorio MV, Ferracin M, Liu CG, et al. MicroRNA gene expression deregulation in human breast cancer[J]. Cancer Res, 2005, 65(16): 7065-7070.
8. Yanaihara N, Caplen N, Bowman E, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis[J]. Cancer Cell, 2006, 9(3): 189-198.
9. Li C, Dong J, Han Z, et al. MicroRNA-219-5p represses the proliferation, migration, and invasion of gastric cancer cells by targeting the LRH-1/Wnt/β-catenin signaling pathway[J]. Oncol Res, 2017, 25(4): 617-627.
10. Rao SA, Arimappamagan A, Pandey P, et al. miR-219-5p inhibits receptor tyrosine kinase pathway by targeting EGFR in glioblastoma[J]. PLoS One, 2013, 8(5): e63164.
11. Huang N, Lin J, Ruan J, et al. MiR-219-5p inhibits hepatocellular carcinoma cell proliferation by targeting glypican-3[J]. FEBS Lett, 2012, 586(6): 884-891.
12. Chen Z, Fillmore CM, Hammerman PS, et al. Non-small-cell lung cancers: a heterogeneous set of diseases[J]. Nat Rev Cancer, 2014, 14(8): 535-546.
13. Zhao J, Guerrero A, Kelnar K, et al. miRNA combination therapy: In vitro anticancer synergy between miR-34a mimic and next generation EGFR tyrosine kinase inhibitors (TKIs) in NSCLC[J]. Cancer Res, 2016, 76(14 Suppl): Abstract nr 4814.
14. Liu C, Kelnar K, Liu B, et al. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44[J]. Nat Med, 2011, 17(2): 211-215.
15. Majid S, Saini S, Dar AA, et al. MicroRNA-205 inhibits Src-mediated oncogenic pathways in renal cancer[J]. Cancer Res, 2011, 71(7): 2611-2621.
16. Valeri N, Braconi C, Gasparini P, et al. MicroRNA-135b promotes cancer progression by acting as a downstream effector of oncogenic pathways in colon cancer[J]. Cancer Cell, 2014, 25(4): 469-483.
17. Zheng X, Jiang F, Katakowski M, et al. ADAM17 promotes breast cancer cell malignant phenotype through EGFR-PI3K-AKT activation[J]. Cancer Biol Ther, 2009, 8(11): 1045-1054.
18. Cui W, Zhang S, Shan C, et al. microRNA-133a regulates the cell cycle and proliferation of breast cancer cells by targeting epidermal growth factor receptor through the EGFR/Akt signaling pathway[J]. FEBS J, 2013, 280(16): 3962-3974.
19. Wang L, Yao J, Shi X, et al. MicroRNA-302b suppresses cell proliferation by targeting EGFR in human hepatocellular carcinoma SMMC-7721 cells[J]. BMC Cancer, 2013, 13(1): 448.
20. Liu L, Shao X, Gao W, et al. MicroRNA-133b inhibits the growth of non-small-cell lung cancer by targeting the epidermal growth factor receptor[J]. FEBS J, 2012, 279(20): 3800-3812.
21. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer[J]. Oncogene, 2007, 26(22): 3291-3310.
22. Gao J, Tian J, Lv Y, et al. Leptin induces functional activation of cyclooxygenase-2 through JAK2/STAT3, MAPK/ERK, and PI3K/AKT pathways in human endometrial cancer cells[J]. Cancer Sci, 2009, 100(3): 389-395.
23. Johnson GL, Lapadat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases[J]. Science, 2002, 298(5600): 1911-1912.
24. Gobeil S, Boucher CC, Nadeau D, et al. Characterization of the necrotic cleavage of poly (ADP-ribose) polymerase (PARP-1): implication of lysosomal proteases[J]. Cell Death Differ, 2001, 8(6): 588-594.
25. Lim YC, Cha YY. Epigallocatechin-3-gallate induces growth inhibition and apoptosis of human anaplastic thyroid carcinoma cells through suppression of EGFR/ERK pathway and cyclin B1/CDK1 complex[J]. J Surg Oncol, 2011, 104(7): 776-780.
26. Lee DH, Szczepanski MJ, Lee YJ. Magnolol induces apoptosis via inhibiting the EGFR/PI3K/Akt signaling pathway in human prostate cancer cells[J]. J Cell Biochem, 2009, 106(6): 1113-1122.
27. Lytle JR, Yario TA, Steitz JA. Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5' UTR as in the 3' UTR[J]. Proc Natl Acad Sci U S A, 2007, 104(23): 9667-9672.
28. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain[J]. PLoS Med, 2005, 2(3): e73.

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