·论著·

沉默HOTAIR通过调控miR-129-5p/EP300轴抑制肾母细胞瘤进展

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摘要 目的 探讨长链非编码RNA（lncRNA）HOX转录反义RNA（HOTAIR）调控miR-129-5p/E1A结合蛋白P300（EP300）轴对肾母细胞瘤（NB）进展的影响。**方法** 选取2022年1月23日至2024年1月23日间我院泌尿外科收治的50例NB患儿，用实时定量PCR检测NB组织及瘤旁组织中HOTAIR、miR-129-5p表达及*EP300* mRNA的表达。将SK-NEP-1细胞分为沉默对照组、HOTAIR沉默组、过表达对照组、miR-129-5p过表达组、HOTAIR沉默+inhibitor NC组、HOTAIR沉默+miR-129-5p inhibitor组、对照组，用实时定量PCR检测各组SK-NEP-1细胞中HOTAIR、miR-129-5p表达及*EP300* mRNA表达；用CCK-8、流式细胞术、Transwell、划痕实验、TUNEL染色分别检测SK-NEP-1细胞增殖、细胞周期、侵袭、迁移和凋亡变化；用双萤光素酶报告基因实验验证HOTAIR与miR-129-5p、miR-129-5p与EP300的关系；采用裸鼠体内移植瘤实验检测瘤体质量与体积。**结果** 与瘤旁组织比较，NB组织中HOTAIR表达及*EP300* mRNA表达升高，miR-129-5p表达降低（$P < 0.05$）。与对照组、沉默对照组比较，HOTAIR沉默组HOTAIR表达及*EP300* mRNA表达、光密度值（OD）$_{450}$值、S期、G_2/M期细胞占比、细胞侵袭数、划痕愈合率、裸鼠体内瘤体质量及体积减少，miR-129-5p表达、G_0/G_1期细胞占比、细胞凋亡率升高（$P < 0.05$）；与对照组、过表达对照组比较，miR-129-5p过表达组*EP300* mRNA表达、OD_{450}值、S期、G_2/M期细胞占比、细胞侵袭数、划痕愈合率、裸鼠体内瘤体质量及体积降低，miR-129-5p表达、G_0/G_1期细胞占比、细胞凋亡率升高（$P < 0.05$）；与HOTAIR沉默组、HOTAIR沉默+inhibitor NC组比较，HOTAIR沉默+miR-129-5p inhibitor组*EP300* mRNA表达、OD_{450}值、S期、G_2/M期细胞占比、细胞侵袭数、划痕愈合率、裸鼠体内瘤体质量及体积增加，miR-129-5p表达、G_0/G_1期细胞占比、细胞凋亡率降低（$P < 0.05$）；HOTAIR与miR-129-5p、miR-129-5p与*EP300*存在靶向调控关系。**结论** 沉默HOTAIR可能通过调控miR-129-5p/EP300轴抑制SK-NEP-1细胞增殖、侵袭、迁移，阻滞细胞周期于G_0/G_1期，诱导细胞凋亡，抑制裸鼠体内肿瘤生长。

关键词 长链非编码RNA；HOX转录反义RNA；miR-129-5p；肾母细胞瘤；E1A结合蛋白P300
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Silencing of HOTAIR inhibits the progression of nephroblastoma via regulating the miR-129-5p/EP300 axis

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Abstract Objective To investigate the effect of long non-coding RNA（lncRNA）HOX transcript antisense RNA（HOTAIR）-based regulation of miR-129-5p/E1A binding protein P300（EP300）axis in nephroblastoma progression. **Methods** Fifty children with nephroblastoma who were admitted to the Department of Urology between January 23，2022 and January 23，2024 were enrolled. The levels of HOTAIR，miR-129-5p，and *EP300* mRNA expression in nephroblastoma and peritumoral tissues were detected by quantitative real time PCR. The SK-NEP-1 cells were divided as follows：silence control，HOTAIR-silenced，overexpression control，miR-129-5p overexpression，HOTAIR-silenced + inhibitor NC，HOTAIR-silenced + miR-129-5p inhibitor，and control groups. The levels of HOTAIR，miR-129-5p，and *EP300* mRNA expression in each group were measured using quantitative real-time PCR. CCK-8 assay，flow cytometry，Transwell assay，wound healing assay and TUNEL staining were used to detect the changes in proliferation，cell cycle，invasion，migration and apoptosis of SK-NEP-1 cells，respectively. Dual-luciferase reporter gene assay was performed to verify the relationships between HOTAIR and miR-129-5p，as well as between miR-129-5p and EP300. The tumor weight and volume were measured in a nude mouse xenograft model. **Results** Compared with peritumoral tissues，in nephroblastoma tissues，the levels of HOTAIR and *EP300* mRNA expression were significantly upregulated，whereas miR-129-5p expression was significantly downregulated（all $P < 0.05$）. Compared with the

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control and silence control groups, the HOTAIR-silenced group had reduced HOTAIR and *EP300* mRNA expression, optical density (OD)$_{450}$ value, percentages of cells in S phase and G_2/M phase, number of invasive cells, wound-healing rate, and tumor weight and volume in nude mice and increased miR-129-5p expression, percentage of cells in G_0/G_1 phase, and apoptosis rate (all $P < 0.05$). Compared with the control and overexpression control groups, in the miR-129-5p overexpression group, the *EP300* mRNA expression, OD_{450} value, percentages of cells in S and G_2/M phases, number of invasive cells, wound-healing rate, and tumor weight and volume in nude mice decreased whereas the miR-129-5p expression, percentage of cells in G_0/G_1 phase, and apoptosis rate increased (all $P < 0.05$). Compared with the HOTAIR-silenced and HOTAIR-silenced + inhibitor NC groups, in the HOTAIR-silenced + miR-129-5p inhibitor group, *EP300* mRNA expression, OD_{450} value, percentages of cells in S phase and G_2/M phase, number of invasive cells, wound-healing rate, and tumor weight and volume in nude mice increased whereas the miR-129-5p expression, percentage of cells in G_0/G_1 phase, and apoptosis rate decreased (all $P < 0.05$). HOTAIR directly targeted miR-129-5p, and miR-129-5p further directly targeted *EP300*. **Conclusion** Silencing HOTAIR potentially inhibits proliferation, invasion, and migration; induces cell-cycle arrest at the G_0/G_1 phase; promotes apoptosis in SK-NEP-1 cells; and suppresses tumor growth in nude mice via regulation of the miR-129-5p/EP300 axis.

Keywords　long non-coding RNA; HOX transcription antisense RNA; miR-129-5p; nephroblastoma; E1A binding protein P300

肾母细胞瘤(nephroblastoma,NB)是儿童泌尿生殖系统最常见的恶性肿瘤。近年来,NB在我国的发病率逐年增加,严重威胁儿童生命[1]。目前,随着手术、放疗、化疗等治疗手段的不断改进,NB患儿的总生存率显著提高[2]。然而,由于NB起病隐匿且进展迅速,15%的患儿仍预后不良[3]。因此,寻找NB儿童的新型诊断标志物和治疗方法至关重要。研究[4]表明,长链非编码RNA(long non-coding RNA,lncRNA)在恶性肿瘤的进展中发挥重要作用。HOX转录反义RNA(HOX transcription antisense RNA,HOTAIR)是一种位于同源框C基因簇中的反式调节lncRNA,其过表达与多种肿瘤的不良预后和复发正相关[5]。已有研究[6]报道,沉默HOTAIR可抑制NB细胞增殖活性并诱导其凋亡,但具体分子机制尚不完全明确。相关研究[7-8]显示,过表达miR-129-5p可抑制透明细胞性肾细胞癌细胞增殖、侵袭和迁移,同时诱导细胞周期停滞在G_0/G_1期并促进细胞凋亡;下调E1A结合蛋白P300(E1A binding protein P300,EP300)抑制了NB细胞的增殖、迁移及侵袭行为。生物信息分析显示,HOTAIR与miR-129-5p、miR-129-5p与EP300轴存在结合位点,但HOTAIR是否可通过调控miR-129-5p/EP300轴参与NB发生尚不清楚。因此,本研究旨在探讨HOTAIR对NB发生发展的影响及分子机制。

1　材料与方法

1.1　材料

1.1.1　组织标本:选取2022年1月23日至2024年1月23日间我院泌尿外科收治的NB患儿50例。收集患儿NB组织及瘤旁组织。所有患儿未接受过放疗或化疗等治疗。本研究获得我院伦理委员会批准(2022DW003),所有患儿家属签署知情同意书。

1.1.2　细胞、实验动物:人NB细胞系SK-NEP-1购自上海沪震生物科技有限公司。6周龄BALB/c裸鼠(雌雄各半)购自凯学生物科技(上海)有限公司,所有动物实验获得我院动物伦理委员会批准(2026DW019)。

1.1.3　试剂与仪器:CCK-8试剂盒(深圳市安培生物科技有限公司),细胞周期检测试剂盒(上海齐一生物科技有限公司),TUNEL细胞凋亡检测试剂盒(上海泽叶生物科技有限公司)。ST-360酶标仪(上海科华生物工程股份有限公司),FACSCalibur流式细胞仪(美国BD公司),CX53光学显微镜(日本OLYMPUS公司),C2-SHS荧光显微镜(日本Nikon公司),BSA 6202S-CW电子天平(德国赛多利斯科学仪器有限公司),16GN游标卡尺(北京德力测控技术有限公司)。

1.2　方法

1.2.1　细胞分组:将SK-NEP-1细胞分为沉默对照组、HOTAIR沉默组、过表达对照组、miR-129-5p过表达组、HOTAIR沉默+inhibitor NC组、HOTAIR沉默+miR-129-5p inhibitor组、对照组。沉默对照组、HOTAIR沉默组、过表达对照组、miR-129-5p过表达组SK-NEP-1细胞分别转染si-NC、si-HOTAIR、mimic NC、miR-129-5p mimic 24 h;HOTAIR沉默+inhibitor NC组SK-NEP-1细胞共转染si-HOTAIR和inhibitor NC 24 h;HOTAIR

沉默+miR-129-5p inhibitor组细胞共转染si-HOTAIR和miR-129-5p inhibitor 24 h；对照组SK-NEP-1细胞不进行转染处理。

1.2.2　HOTAIR、miR-129-5p表达及*EP300* mRNA表达的检测：通过TRIzol试剂提取组织及细胞总RNA。将RNA逆转录为cDNA后，以cDNA为模板进行实时定量PCR反应。miR-129-5p以*U6*为内参基因，HOTAIR、EP300以*GAPDH*为内参基因，采用$2^{-\Delta\Delta Ct}$法计算基因表达量。引物序列如下：*GAPDH*，正向5'-GGGAAACTGTGGCGTGAT-3'，反向5'-GAGTGGGTGTCGCTGTTGA-3'；HOTAIR，正向5'-GAGTCTGATGTTTACAAGACC-3'，反向5'-CAACGAGCTTATAAGGAAGG-3'；*EP300*，正向5'-GCAGCATCACATGCAACAGA-3'，反向5'-AGGAGTCGCTGCTGATAGGC-3'；*U6*，正向5'-CAAGGATGACACGCAAATTC-3'，反向5'-AAAAATGGAACGCTTCACGA-3'；miR-129-5p，正向5'-CGCTTTTTGCGGTCTGG-3'，反向5'-AGTGCAGGGTCCGAGGTATT-3'。

1.2.3　细胞增殖检测：将SK-NEP-1细胞接种至96孔板（1×10^5/孔），按照1.2.1所述进行对应处理后，加入CCK-8溶液（10 μL/孔），培养2 h后，利用酶标仪测定光密度（optical density，OD）值。

1.2.4　细胞周期检测：收集各组细胞，用1 mL 70%冷乙醇固定2 h，用预冷磷酸盐缓冲液（phosphate-buffered saline，PBS）洗涤细胞3次，室温下用100 μL PBS和0.5 mL 碘化丙啶（propidium iodide，PI）染色缓冲液悬浮细胞15 min。用流式细胞仪测定细胞周期分布。

1.2.5　细胞侵袭检测：用无血清DMEM培养液悬浮各组细胞浓度至4×10^4/mL，取0.1 mL细胞悬液置于基质胶包被的Transwell上室中，再向下室填充DMEM培养液（含20%胎牛血清）孵育24 h后，收集上室并清洗。用4%多聚甲醛在25 ℃下固定侵袭细胞15 min，0.3%结晶紫溶液孵育30 min。光学显微镜下观察。

1.2.6　细胞迁移检测：将各组细胞培养于6孔板（1×10^5/孔）中，当细胞融合达70%~80%时，将培养基替换为无血清DMEM，细胞孵育过夜。随后，用200 μL无菌吸管尖刮拭细胞单层。PBS冲洗后的细胞培养24 h。通过光学显微镜在0和24 h采集图像。使用ImageJ软件确定每张图片的划痕愈合面积，最后计算划痕愈合率。

1.2.7　细胞凋亡检测：各组细胞用PBS洗涤3次，4%多聚甲醛固定15 min，再将细胞置于0.15% 曲拉通X-100中孵育5 min。加入TUNEL混合液在37 ℃下孵育60 min。细胞核用4'，6-二脒基-2-苯基吲哚（0.5 μg/mL）染色5 min。使用荧光显微镜观察细胞凋亡。

1.2.8　靶向关系验证：将包含miR-129-5p结合位点的HOTAIR、*EP300*序列亚克隆并插入到pmirGLO载体中，构建HOTAIR、*EP300*野生型（wild type，WT）和突变型（mutant type，MUT）报告质粒，并依次命名为HOTAIR-WT、HOTAIR-MUT、*EP300*-WT、*EP300*-MUT。将上述质粒分别依次与mimic NC或miR-129-5p mimic共转染于SK-NEP-1细胞，48 h后检测双萤光素酶活性变化。

1.2.9　裸鼠移植瘤实验：将1.2.1中的各组SK-NEP-1细胞浓度调至1×10^7/mL，分别按0.2 mL/只接种于裸鼠左前上肢腋下处皮下，并依次命名为Vivo-沉默对照组、Vivo-HOTAIR沉默组、Vivo-过表达对照组、Vivo-miR-129-5p过表达组、Vivo-HOTAIR沉默+inhibitor NC组、Vivo-HOTAIR沉默+miR-129-5p inhibitor组、Vivo-对照组，每组6只。21 d后采用颈椎脱臼法处死全部裸鼠，剥离瘤体，测量瘤体体积、称量瘤体质量，瘤体体积=1/2×瘤体最大直径×（瘤体最小直径）^{2}。

1.3　统计学分析

采用SPSS 22.0进行统计分析。数据以$\bar{x}\pm s$表示，2组比较采用*t*检验，组间比较采用单因素方差分析和事后SNK-*q*多重比较检验。$P<0.05$为差异有统计学意义。

2　结果

2.1　NB组织和瘤旁组织中HOTAIR、miR-129-5p表达及*EP300* mRNA表达比较

与瘤旁组织比较，NB组织HOTAIR表达及*EP300* mRNA升高，miR-129-5p表达降低（$P<0.05$），见表1。

2.2　HOTAIR沉默或miR-129-5p过表达对SK-NEP-1细胞中HOTAIR、miR-129-5p表达及*EP300* mRNA的影响

与对照组、沉默对照组比较，HOTAIR沉默组HOTAIR表达及*EP300* mRNA表达降低，miR-129-5p表达升高（$P<0.05$）；与对照组、过表达对照组比较，miR-129-5p过表达组miR-129-5p表达升高，*EP300*

mRNA表达降低（$P < 0.05$）；与HOTAIR沉默组、HOTAIR沉默+inhibitor NC组比较，HOTAIR沉默+miR-129-5p inhibitor组miR-129-5p表达降低，*EP300* mRNA表达升高（$P < 0.05$），见表2。

表1　NB组织及瘤旁组织HOTAIR、miR-129-5p表达及*EP300* mRNA表达变化

Tab.1　Levels of HOTAIR，miR-129-5p，and *EP300* mRNA expression in nephroblastoma and peritumoral tissues

Tissue	*n*	HOTAIR	miR-129-5p	*EP300* mRNA
Paratumor	50	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
NB	50	2.26 ± 0.17[1)]	0.32 ± 0.03[1)]	1.85 ± 0.14[1)]

1）$P < 0.05$ vs. paratumor tissues.

表2　各组SK-NEP-1细胞HOTAIR、miR-129-5p表达及*EP300* mRNA比较

Tab.2　Intergroup comparison of HOTAIR，miR-129-5p，and *EP300* mRNA expression in SK-NEP-1 cells

Group	*n*	HOTAIR	miR-129-5p	*EP300* mRNA
Control	6	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Silence control	6	1.01 ± 0.01	0.99 ± 0.02	1.02 ± 0.01
HOTAIR silenced	6	0.25 ± 0.02[1),2)]	1.95 ± 0.11[1),2)]	0.38 ± 0.03[1),2)]
Overexpression control	6	1.02 ± 0.01	0.98 ± 0.02	1.00 ± 0.01
miR-129-5p overexpression	6	1.00 ± 0.02	2.12 ± 0.14[1),3)]	0.31 ± 0.02[1),3)]
HOTAIR silenced+inhibitor NC	6	0.23 ± 0.02	1.93 ± 0.13	0.40 ± 0.03
HOTAIR silenced+miR-129-5p inhibitor	6	0.22 ± 0.02	1.21 ± 0.10[4),5)]	0.82 ± 0.06[4),5)]

1）$P < 0.05$ vs. control group；2）$P < 0.05$ vs. silence control group；3）$P < 0.05$ vs. overexpression control group；4）$P < 0.05$ vs. HOTAIR silenced group；5）$P < 0.05$ vs. HOTAIR silenced + inhibitor NC group.

2.3　HOTAIR沉默或miR-129-5p过表达对SK-NEP-1细胞增殖和细胞周期的影响

与对照组、沉默对照组比较，HOTAIR沉默组OD_{450}值及S期、G_2/M期细胞占比降低，G_0/G_1期细胞占比升高（$P < 0.05$）；与对照组、过表达对照组比较，miR-129-5p过表达组对应指标变化趋势与上述一致（$P < 0.05$）；与HOTAIR沉默组、HOTAIR沉默+inhibitor NC组比较，HOTAIR沉默+miR-129-5p inhibitor组OD_{450}值、S期、G_2/M期细胞占比升高，G_0/G_1期细胞占比降低（$P < 0.05$），见表3。

表3　各组SK-NEP-1细胞OD_{450}值及G_0/G_1期、S期、G_2/M期细胞占比比较

Tab.3　Intergroup comparison of the OD_{450} value and cell percentages in G_0/G_1，S，and G_2/M phases of SK-NEP-1 cells

Group	*n*	OD_{450} value	G_0/G_1 phase (%)	S phase (%)	G_2/M phase (%)
Control	6	1.23 ± 0.11	58.81 ± 2.67	21.69 ± 1.08	19.50 ± 0.83
Silence control	6	1.25 ± 0.13	59.02 ± 2.71	21.72 ± 1.10	19.26 ± 0.93
HOTAIR silenced	6	0.46 ± 0.04[1),2)]	77.65 ± 3.46[1),2)]	9.36 ± 0.38[1),2)]	12.99 ± 1.15[1),2)]
Overexpression control	6	1.21 ± 0.10	60.15 ± 2.83	21.71 ± 1.06	18.14 ± 0.92
miR-129-5p overexpression	6	0.39 ± 0.03[1),3)]	79.85 ± 3.43[1),3)]	9.04 ± 0.37[1),3)]	11.11 ± 0.58[1),3)]
HOTAIR silenced+inhibitor NC	6	0.43 ± 0.03	78.22 ± 3.67	9.40 ± 0.39	12.38 ± 0.61
HOTAIR silenced+miR-129-5p inhibitor	6	0.89 ± 0.08[4),5)]	65.56 ± 3.18[4),5)]	15.83 ± 0.64[4),5)]	18.61 ± 0.92[4),5)]

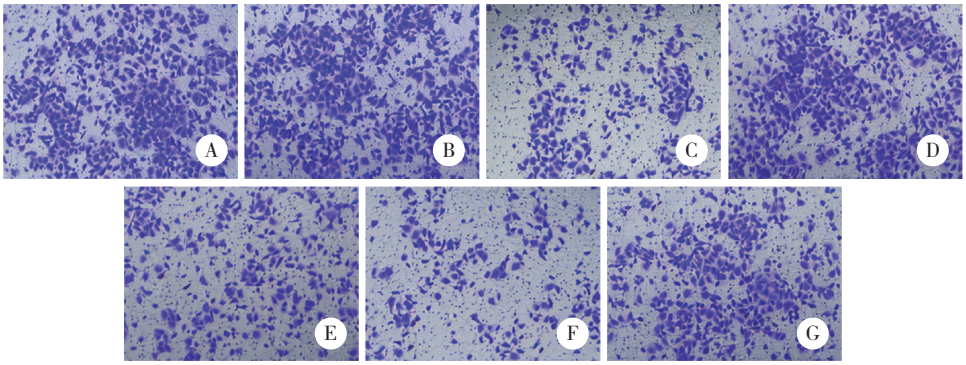
1）$P < 0.05$ vs. control group；2）$P < 0.05$ vs. silence control group；3）$P < 0.05$ vs. overexpression control group；4）$P < 0.05$ vs. HOTAIR silenced group；5）$P < 0.05$ vs. HOTAIR silenced + inhibitor NC group.

2.4　HOTAIR沉默或miR-129-5p过表达对SK-NEP-1细胞侵袭和迁移的影响

与对照组、沉默对照组比较，HOTAIR沉默组细胞侵袭数、划痕愈合率降低（$P < 0.05$）；与对照组、

过表达对照组比较，miR-129-5p过表达组细胞侵袭数、划痕愈合率降低（$P < 0.05$）；与HOTAIR沉默组、HOTAIR沉默+inhibitor NC组比较，HOTAIR沉默+miR-129-5p inhibitor组细胞侵袭数、划痕愈合率升高（$P < 0.05$），见图1、2和表4。

2.5　HOTAIR沉默或miR-129-5p过表达对SK-NEP-1细胞凋亡的影响



A，control group；B，silence control group；C，HOTAIR silenced group；D，overexpression control group；E，miR-129-5p overexpression group；F，HOTAIR silenced+inhibitor NC group；G，HOTAIR silenced+miR-129-5p inhibitor.

图1　SK-NEP-1细胞侵袭Transwell检测结果　×200

Fig.1　Results of the Transwell assay for ascertaining SK-NEP-1 cell invasion　×200

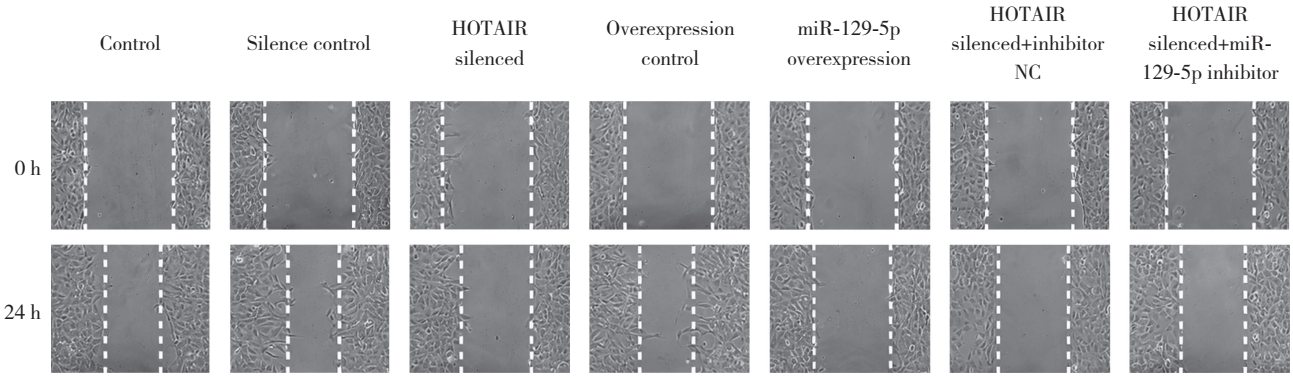


图2　SK-NEP-1细胞迁移划痕实验　×200

Fig.2　Wound-healing assay for SK-NEP-1 cell migration　×200

表4　各组SK-NEP-1细胞侵袭数、划痕愈合率比较

Tab.4　Comparison of the number of invasive cells and wound-healing rate of SK-NEP-1 cells in each group

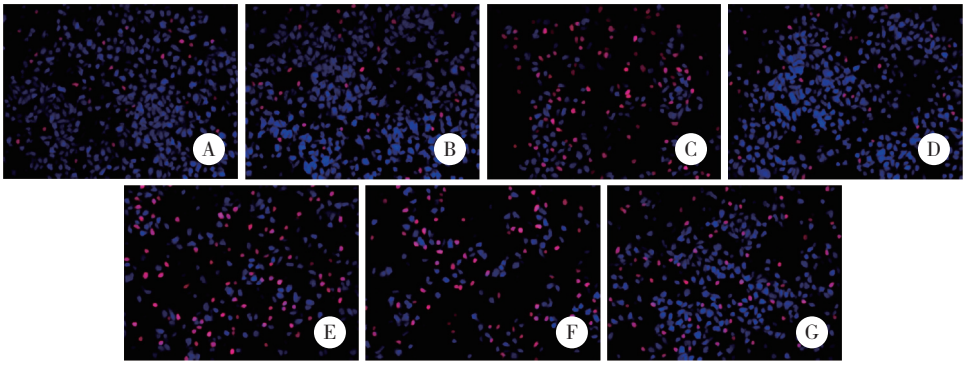
Group	n	Number of invasive cells	Wound healing rate (%)
Control	6	86.67 ± 4.12	38.65 ± 1.83
Silence control	6	88.23 ± 4.15	39.12 ± 1.95
HOTAIR silenced	6	39.66 ± 1.97[1),2)]	17.65 ± 0.82[1),2)]
Overexpression control	6	87.93 ± 4.19	39.23 ± 1.87
miR-129-5p overexpression	6	35.58 ± 1.69[1),3)]	14.23 ± 0.65[1),3)]
HOTAIR silenced+inhibitor NC	6	40.33 ± 2.01	18.11 ± 0.92
HOTAIR silenced+miR-129-5p inhibitor	6	65.56 ± 3.05[4),5)]	29.55 ± 1.43[4),5)]

1) $P < 0.05$ vs. control group；2) $P < 0.05$ vs. silence control group；3) $P < 0.05$ vs. overexpression control group；4) $P < 0.05$ vs. HOTAIR silenced group；5) $P < 0.05$ vs. HOTAIR silenced + inhibitor NC group.

与对照组（5.67% ± 0.23%）、沉默对照组（5.71% ± 0.25%）比较，HOTAIR沉默组（36.69% ± 1.81%）细胞凋亡率升高（$P < 0.05$）；与对照组、过表达对照组

(5.76% ± 0.25%)比较,miR-129-5p过表达组(40.41% ± 2.07%)细胞凋亡率升高($P < 0.05$);与HOTAIR沉默组、HOTAIR沉默+inhibitor NC组(36.72% ± 1.83%)比较,HOTAIR沉默+miR-129-5p inhibitor组(19.36% ± 0.81%)细胞凋亡率降低($P < 0.05$),见图3。

2.6　HOTAIR靶向调控miR-129-5p/EP300轴



A, control group; B, silence control group; C, HOTAIR silenced group; D, overexpression control group; E, miR-129-5p overexpression group; F, HOTAIR silenced+inhibitor NC group; G, HOTAIR silenced+miR-129-5p inhibitor.

图3　SK-NEP-1细胞凋亡的TUNEL染色检测 ×200

Fig.3　TUNEL staining assay for determining SK-NEP-1 cell apoptosis ×200

HOTAIR与miR-129-5p、miR-129-5p与*EP300*存在结合位点,见图4。miR-129-5p mimic和HOTAIR-WT共转染组的萤光素酶活性(0.23 ± 0.02)低于mimic NC和HOTAIR-WT共转染组(1.00 ± 0.02,$P < 0.05$);miR-129-5p mimic和*EP300*-WT共转染组的萤光素酶活性(0.16 ± 0.02)低于mimic NC和*EP300*-WT共转染组(0.99 ± 0.04,$P < 0.05$)。

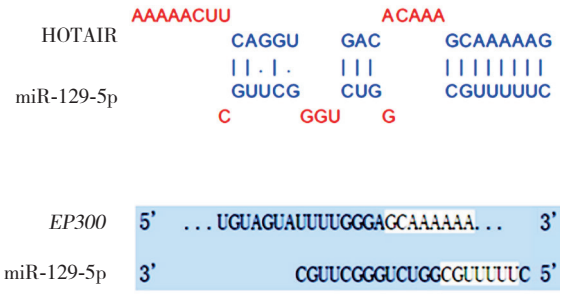


图4　结合位点图

Fig.4　Diagram of the binding sites

2.7　HOTAIR沉默或miR-129-5p过表达对裸鼠体内移植瘤生长的影响

与Vivo-对照组、Vivo-沉默对照组比较,Vivo-HOTAIR沉默组瘤体质量及体积降低($P < 0.05$);与Vivo-对照组、Vivo-过表达对照组比较,Vivo-miR-129-5p过表达组瘤体质量及体积减少($P < 0.05$);与Vivo-HOTAIR沉默组、Vivo-HOTAIR沉默+inhibitor NC组比较,Vivo-HOTAIR沉默+miR-129-5p inhibitor组瘤体质量及体积增加($P < 0.05$),见表5。

3　讨论

HOTAIR是多种恶性肿瘤的促癌基因。如过表达HOTAIR可促进结肠腺癌细胞的增殖和侵袭[9]。本研究发现,NB组织中HOTAIR表达高于瘤旁组织,表明HOTAIR在NB中发挥致癌作用。选取人NB细胞SK-NEP-1细胞为研究对象,并对其进行HOTAIR沉默处理,结果发现沉默HOTAIR可通过抑制细胞增殖、侵袭、迁移,将细胞周期阻滞于G_0/G_1期,诱导细胞凋亡的方式发挥抗NB作用。体内裸鼠移植瘤实验也证明了沉默HOTAIR能够减缓肿瘤生长。提示HOTAIR可能是治疗NB的潜在有效靶点之一。

目前已有HOTAIR调节miR-129-5p影响肿瘤进展的研究[10]。本研究证实了miR-129-5p为HOTAIR的靶基因。miR-129-5p在多种肿瘤中表达失调,过表达miR-129-5p可抑制透明细胞性肾细胞癌细胞活力和迁移率[11]。本研究中,miR-129-5p在NB组织中表达下调,过表达miR-129-5p抑制了SK-NEP-1细胞恶性行为及裸鼠体内肿瘤生长,表明miR-129-5p在NB中具有抑癌作用。此外,沉默HOTAIR可上调SK-NEP-1细胞中miR-129-5p表达,而过表达miR-129-5p对SK-NEP-1细胞中HOTAIR表达的影响差异无统计学意义,说明HOTAIR可调控miR-129-5p表达,但miR-129-5p不能调控HOTAIR表达,推测沉

默HOTAIR可能通过上调miR-129-5p表达抑制SK-NEP-1细胞生物学行为及裸鼠体内肿瘤生长。为验证推测，本研究用miR-129-5p inhibitor进行了回复实验，结果显示，miR-129-5p inhibitor逆转了沉默HOTAIR对SK-NEP-1细胞生物学行为及裸鼠体内肿瘤生长的影响。

表5　各组裸鼠体内瘤体质量及体积变化比较

Tab.5　Intergroup comparison of tumor weight and volume changes in nude mice

Group	*n*	Tumor weight (g)	Tumor volume (mm^3)
Vivo-control	6	0.68 ± 0.05	1 307.69 ± 61.22
Vivo-silence control	6	0.70 ± 0.06	1 346.15 ± 60.57
Vivo-HOTAIR silenced	6	0.32 ± 0.03[1),2)]	615.38 ± 30.25[1),2)]
Vivo-overexpression control	6	0.71 ± 0.07	1 365.38 ± 61.38
Vivo-miR-129-5p overexpression	6	0.25 ± 0.02[1),3)]	480.77 ± 21.25[1),3)]
Vivo-HOTAIR silenced+inhibitor NC	6	0.33 ± 0.03	634.62 ± 30.39
Vivo-HOTAIR silenced+miR-129-5p inhibitor	6	0.45 ± 0.04[4),5)]	865.38 ± 43.39[4),5)]

1) $P < 0.05$ vs. Vivo-control group; 2) $P < 0.05$ vs.Vivo-silence control group; 3) $P < 0.05$ vs. Vivo-overexpression control group; 4) $P < 0.05$ vs. Vivo-HOTAIR silenced group; 5) $P < 0.05$ vs. Vivo-HOTAIR silenced + inhibitor NC group.

miRNA通过与靶标mRNA的3'–非翻译区结合，在转录后调节其靶标基因表达[12-13]。本研究确定了*EP300*为miR-129-5p的靶基因。EP300是一种在细胞增殖和分化中扮演重要角色的转录共激活因子[14]。研究[8]报道，EP300在NB组织中处于显著上调状态；沉默*EP300*减弱了NB细胞的增殖、迁移及侵袭行为。本研究发现，*EP300* mRNA在NB组织中高表达，沉默HOTAIR可上调SK-NEP-1细胞中miR-129-5p表达，下调*EP300* mRNA表达，miR-129-5p过表达亦可抑制*EP300* mRNA表达，且miR-129-5p inhibitor逆转了沉默HOTAIR对SK-NEP-1细胞中*EP300* mRNA表达的抑制作用，表明沉默HOTAIR可能通过调控miR-129-5p/EP300轴抑制SK-NEP-1细胞生物学行为及裸鼠体内肿瘤生长。

综上所述，沉默HOTAIR抑制SK-NEP-1细胞生物学行为及裸鼠体内肿瘤生长的机制可能与调控miR-129-5p/EP300轴有关。本研究可能为NB的治疗提供新的靶点。

参考文献：

[1] CHANG MM, LI D, SU L, et al. Nephroblastoma-specific dysregulated gene SNHG15 with prognostic significance: scRNA-Seq with bulk RNA-Seq data and experimental validation [J]. Discov Oncol, 2024, 15(1): 87. DOI: 10.1007/s12672-024-00946-w.

[2] HE YL, LIU JY, TIAN YJ, et al. miR-30a-5p regulates the proliferation of wilms tumor cells through CyclinE2 [J]. Int J Urol, 2026, 33(1): e70270. DOI: 10.1111/iju.70270.

[3] LUO B, FENG SS, LI TL, et al. Transcription factor HOXB2 upregulates NUSAP1 to promote the proliferation, invasion and migration of nephroblastoma cells via the PI3K/Akt signaling pathway [J]. Mol Med Rep, 2022, 25(6): 205-214. DOI: 10.3892/mmr.2022.12721 DOI: 10.3892/mmr.2022.12721.

[4] ZHU QL, ZHAN DM, YANG YG, et al. LINC00173 promotes Wilms' tumor progression through MGAT1-mediated MUC3A N-glycosylation [J]. Cell Cycle, 2022, 21(17): 1795-1810. DOI: 10.1080/15384101.2022.2070399.

[5] CHU DX, JIN Y, WANG BR, et al. LncRNA HOTAIR enhances epithelial-to-mesenchymal transition to promote the migration and invasion of liver cancer by regulating NUAK1 via epigenetic inhibition miR-145-5p expression [J]. J Cancer, 2023, 14(12): 2329-2343. DOI: 10.7150/jca.85335.

[6] 杨莉莉，曹广海，刘玉洁，等. 长链非编码RNA HOTAIR通过Wnt/β-catenin信号通路对肾母细胞瘤细胞增殖凋亡及耐药的影响[J]. 中华肿瘤杂志，2021，43(7)：769-774. DOI：10.3760/cma.j.cn112152-20191227-00846.

[7] GAO B, WANG LJ, ZHANG N, et al. miR-129-5p inhibits clear cell renal cell carcinoma cell proliferation, migration and invasion by targeting SPN [J]. Cancer Cell Int, 2021, 21(1): 263. DOI: 10.1186/s12935-021-01820-3.

[8] CAO J, LIU GS, ZOU NZ, et al. microRNA-200c-3p suppresses proliferation and invasion of nephroblastoma cells by targeting EP300 and inactivating the AKT/FOXO1/p27 pathway [J]. Neoplasma, 2022, 1(1): 210922N1340. DOI: 10.4149/neo_2022_210922N1340.

[9] GONG NN, LI F, WANG YN, et al. HOXC11 positively regulates the long non-coding RNA HOTAIR and is associated with poor prognosis in colon adenocarcinoma [J]. Exp Ther Med, 2021, 22(5): 1310. DOI: 10.3892/etm.2021.10745.

[10] WU DD, ZHU J, FU Y, et al. LncRNA HOTAIR promotes breast cancer progression through regulating the miR-129-5p/FZD7 axis [J]. Cancer Biomark, 2021, 30(2): 203-212. DOI: 10.3233/CBM-190913.

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中的作用研究[J]. 中国妇产科临床杂志,2022,23(2):174-178. DOI:10.13390/j.issn.1672-1861.2022.02.018.
[2] 曾克非,夏婷婷,吴小兰,等. 木犀草素通过TLR4/PI3K/AKT通路对子痫前期大鼠胎盘血管生成及细胞凋亡的影响[J]. 中国免疫学杂志,2024,40(2):330-336. DOI:10.3969/j.issn.1000-484X.2024.02.019.
[3] 张金环,夏艳秋,孙静莉. 槲皮素通过上调SIRT1减轻子痫前期大鼠胎盘的炎性反应[J]. 解剖科学进展,2023,29(2):127-130,134. DOI:10.16695/j.cnki.1006-2947.2023.02.005.
[4] 乐宁,杨琪,音金萍,等. 健脾益气方通过抑制DEN肝癌大鼠肝组织NLRP3炎性小体的激活影响血清炎症因子水平[J]. 基因组学与应用生物学,2022,41(6):1350-1359. DOI:10.13417/j.gab.041.001350.
[5] MOHAMED KAMEL GA,HARAHSHEH E,HUSSEIN S. Diacerein ameliorates acetaminophen hepatotoxicity in rats via inhibiting HMGB1/TLR4/NF-κB and upregulating PPAR-γ signal[J]. Mol Biol Rep,2022,49(7):5863-5874. DOI:10.1007/s11033-022-07366-5.
[6] FAN MH,LI XB,GAO XL,et al. LPS induces preeclampsia-like phenotype in rats and HTR8/SVneo cells dysfunction through TLR4/p38 MAPK pathway[J]. Front Physiol,2019,10:1030. DOI:10.3389/fphys.2019.01030.
[7] KıZıLASLAN N,AYDıN D,SUMBUL O,et al. The effect of quercetin on absence epilepsy in WAG/Rij rats[J]. Neurol Res,2023,45(8):701-707. DOI:10.1080/01616412.2023.2194182.
[8] WU YY,KANG F,YANG YY,et al. The protective effect of magnesium sulfate on placental inflammation via suppressing the NF-κB pathway in a preeclampsia-like rat model[J]. Pregnancy Hypertens,2023,31:4-13. DOI:10.1016/j.preghy.2022.11.004.
[9] QIN ZM,LONG Y. Immunological dysregulation in preeclampsia:pathogenesis and clinical implications[J]. Int Immunopharmacol,2025,163:115314. DOI:10.1016/j.intimp.2025.115314.
[10] 卢慧华,胡小英. 槲皮素对大鼠子痫前期模型血管内皮损伤及Nrf2/ARE信号通路的影响[J]. 中国优生与遗传杂志,2021,29(2):190-193. DOI:10.13404/j.cnki.cjbhh.20210625.027.
[11] 刘意,何佳佳. 子痫前期并发胎儿生长受限预测研究进展[J]. 临床医学进展,2022,12(3):2334-2340. DOI:10.12677/ACM.2022.123336.
[12] ORABONA R,SCIATTI E,SARTORI E,et al. The impact of preeclampsia on women's health:cardiovascular long-term implications[J]. Obstet Gynecol Surv,2020,75(11):703-709. DOI:10.1097/ogx.0000000000000846.
[13] 彭瑞妮,刘犇,余四九. 运输应激对山羊免疫器官细胞凋亡及Bcl-2和Bax表达的影响[J]. 农业生物技术学报,2022,30(4):676-685. DOI:10.3969/j.issn.1674-7968.2022.04.006.
[14] QI BC,ZHENG Y,GAO WQ,et al. Alpha-lipoic acid impedes myocardial ischemia-reperfusion injury,myocardial apoptosis,and oxidative stress by regulating HMGB1 expression[J]. Eur J Pharmacol,2022,933:175295. DOI:10.1016/j.ejphar.2022.175295.
[15] HE LD,SONG QF,HU JF,et al. Expression of HMGB1-TLR4 in placentas from preeclamptic pregnancies and its effect on proliferation and invasion of HTR-8/SVneo cells[J]. Gynecol Obstet Invest,2023,88(3):159-167. DOI:10.1159/000530006.

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[11] WU ZP,CHEN DM,WANG K,et al. Long non-coding RNA SNHG12 functions as a competing endogenous RNA to regulate MDM4 expression by sponging miR-129-5p in clear cell renal cell carcinoma[J]. Front Oncol,2019,9:1260. DOI:10.3389/fonc.2019.01260.
[12] 李富博,李爱科,饶井芬,等. lncRNA FGD5-AS1靶向miR-512-3p/RAB31抑制膀胱癌细胞增殖、侵袭和上皮–间质转化[J]. 中国医科大学学报,2026,55(1):33-40. DOI:10.12007/j.issn.0258-4646.2026.01.006.
[13] LU XJ,GAO WW,LI JC,et al. miRNA-381 regulates renal cancer stem cell properties and sunitinib resistance via targeting SOX4[J]. Biochem Biophys Rep,2023,36:101566. DOI:10.1016/j.bbrep.2023.101566.
[14] KIM KB,KABRA A,KIM DW,et al. KIX domain determines a selective tumor-promoting role for EP300 and its vulnerability in small cell lung cancer[J]. Sci Adv,2022,8(7):eabl4618. DOI:10.1126/sciadv.abl4618.

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