miR-106b-5p靶向调控SIRT7/SMAD4信号通路对口腔鳞状细胞癌侵袭和迁移的影响机制探究

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摘要 该文探讨了miR-106b-5p对口腔鳞状细胞癌(OSCC)细胞迁移和侵袭的影响及其机制。采用qRT-PCR检测OSCC组织与细胞系中miR-106b-5p表达情况, 将SCC15、OECM1细胞分为Control组、miR-NC组、miR-106b-5p mimics组、anti-miR-NC组、anti-miR-106b-5p、anti-miR-106b-5p+si-NC组、anti-miR-106b-5p+si-SIRT7组, 分别检测各组细胞增殖、迁移及侵袭能力, Western blot检测E-cadherin、N-cadherin、MMP-9、SIRT7、SMAD4蛋白表达情况, 双荧光素酶报告基因实验与RIP实验验证miR-106b-5p与*SIRT7*的靶向关系。结果显示, miR-106b-5p在OSCC组织与细胞中表达水平升高(P<0.05), 过表达miR-106b-5p可显著促进OSCC细胞增殖、迁移、侵袭及EMT, 抑制miR-106b-5p表达可显著抑制OSCC细胞增殖、迁移、侵袭及EMT(P<0.05); 双荧光素酶报告基因实验与RIP实验证实, miR-106b-5p与*SIRT7*存在靶向关系(P<0.05); 抑制SIRT7表达可逆转抑制miR-106b-5p表达对OSCC细胞增殖、迁移、侵袭及EMT的抑制作用(P<0.05)。总之, miR-106b-5p在OSCC组织与细胞中上调表达, 抑制miR-106b-5p表达可通过调节SIRT7/SMAD4信号通路, 抑制OSCC细胞增殖、迁移、侵袭及EMT。

关键词 微小RNA-106b-5p; 口腔鳞状细胞癌; 迁移; 侵袭; SIRT7; SMAD4

Mechanism of miR-106b-5p on Invasion and Migration of Oral Squamous Cell Carcinoma through Targeted Regulation of SIRT7/SMAD4 Signaling Pathway

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Abstract This study aimed to investigate the effects and mechanism of miR-106b-5p on migration and invasion of OSCC (oral squamous cell carcinoma) cells. The expression of miR-106b-5p in OSCC tissues and cell lines was detected by qRT-PCR. SCC15 and OECM1 cells were divided into Control group, miR-NC group, miR-106b-5p mimics group, anti-miR-NC group, anti-miR-106b-5p group, anti-miR-106b-5p+si-NC group, and anti-miR-106b-5p+si-SIRT7 group, respectively. Cell proliferation, migration and invasion were detected in each group; Western blot was used to detect the protein expression of E-cadherin, N-cadherin, MMP-9, SIRT7 and SMAD4;

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double luciferase reporter gene assay and RIP assay verified the targeting relationship between miR-106b-5p and *SIRT7*. The results showed that the expression levels of miR-106b-5p in OSCC tissues and cells were increased (P<0.05). Overexpression of miR-106b-5p could significantly promote the proliferation, migration, invasion and EMT of OSCC cells, and inhibition of miR-106b-5p expression could significantly inhibit the proliferation, migration, invasion and EMT of OSCC cells (P<0.05). Double luciferase reporter gene experiment and RIP experiment confirmed that there was a targeting relationship between miR-106b-5p and *SIRT7* (P<0.05). Inhibition of SIRT7 expression could reverse the inhibitory effects of miR-106b-5p expression on the proliferation, migration, invasion and EMT of OSCC cells (P<0.05). In conclusion, the expression of miR-106b-5p is up-regulated in OSCC tissues and cells. Inhibition of miR-106b-5p expression can inhibit the proliferation, migration, invasion and EMT of OSCC cells by regulating SIRT7/SMAD4 signal pathway.

Keywords miR-106b-5p; oral squamous cell carcinoma; migration; invasion; SIRT7; SMAD4

口腔鳞状细胞癌(oral squamous cell carcinoma, OSCC)是口腔癌的主要形式，占所有口腔恶性肿瘤的90%以上，由于肿瘤易发生转移与侵袭，导致患者5年生存率不足50%[1-2]。因此，研究OSCC的发病分子机制，对寻找新型靶向治疗策略具有重要意义。微小RNA(microRNA, miRNA)表达失调与OSCC的迁移与侵袭密切相关，已成为OSCC药物干预的新靶点[3-4]。据报道，miR-106b-5p在食管鳞状细胞癌、结直肠癌中上调表达，抑制miR-106b-5p表达可抑制肿瘤细胞增殖、迁移与侵袭[5-6]。然而，miR-106b-5p在OSCC中的作用尚不清楚。沉默信息调节因子2同源蛋白7(silencing information regulator 2 homologous protein 7, SIRT7)通过调节不同信号通路参与肿瘤发生发展，研究显示，SIRT7在OSCC中表达下调，可通过调控SMAD4表达，调节OSCC细胞上皮–间充质转化(epithelial-mesenchymal transition, EMT)及迁移[7]。生物信息学(StarBase、targetscan)分析显示，miR-106b-5p与*SIRT7*存在结合位点，推测miR-106b-5p可能通过调节SIRT7/SMAD4影响OSCC细胞的迁移与侵袭。本研究将探索miR-106b-5p对OSCC细胞增殖、迁移、侵袭及SIRT7/SMAD4通路的影响，为OSCC的靶向治疗提供参考。

1 材料与方法

1.1 细胞与主要试剂

HOK及SCC15、CAL27、HSC3、SCC9、OECM1购自美国ATCC细胞库；miR-106b-5p模拟物(miR-106b-5p mimics, 5′-UAA AGU GCU GAC AGU GCA GAU-3′)及对照(miR-NC, 5′-UUC UCC GAA CGU GUC ACG UTT-3′)、miR-106b-5p inhibitor(anti-miR-106b-5p, 5′-AUC UGC ACU GUC AGC ACU UUA-3′)及对照(anti-miR-NC, 5′-CAG UAC UUU UGU GUA GUA CAA-3′)、SIRT7小干扰RNA(si-SIRT7, 5′-GTG GAC ACT GCT TCA GAA A-3′)及对照(si-NC, 5′-CCU UGC GUG GGA GCG CGA A-3′)、miR-106b-5p及*U6*引物购自广州锐博生物科技有限公司；TRIzol(总RNA提取试剂)(YT526)、SYBR Green qPCR预混液(ALH185)、双荧光素酶报告基因检测试剂盒(KFS303)、CCK-8细胞活力检测试剂盒(SNM507)购自北京百奥莱博科技有限公司；兔抗人E-cadherin(AG1544)、N-cadherin(AG1554)、MMP-9(AF5234)、SIRT7(AF7986)、SMAD4(AG2569)及β-actin(AF5003)抗体购自上海碧云天生物技术有限公司。

1.2 方法

1.2.1 标本来源 本研究经过海南医学院第一附属医院伦理委员会批准(审批号20190301)。收集2019年5月至2022年3月在本院进行手术切除的患者OSCC组织及癌旁组织43例，患者在术前未进行放化疗等治疗，其中男25例，女18例，年龄为36~72岁，平均(50.21±12.46)岁，经病理诊断为OSCC，qRT-PCR检测标本中miR-106b-5p表达。本研究所有患者均签署书面知情同意书。

1.2.2 细胞转染及分组 当HOK及SCC15、CAL27、HSC3、SCC9、OECM1细胞生长至对数期时，提取细胞总RNA，检测其miR-106b-5p表达水平。

将对数生长期的SCC15、OECM1细胞接种于6孔板中，根据质粒转染情况，将细胞分为Control组(正常培养，不转染)、miR-NC组(转染miR-NC)、miR-106b-5p mimics组(转染miR-106b-5p mimics)、

anti-miR-NC组(转染anti-miR-NC)、anti-miR-106b-5p(转染anti-miR-106b-5p)、anti-miR-106b-5p+si-NC组(共转染anti-miR-106b-5p和si-NC)、anti-miR-106b-5p+si-SIRT7组(共转染anti-miR-106b-5p和si-SIRT7), 每组设置6个复孔, 转染48 h后检验转染效率, 用于后续实验。

1.2.3 qRT-PCR法检测miR-106b-5p表达水平 TRIzol试剂提取总RNA, 检测RNA的纯度和浓度, 使用逆转录试剂盒制备cDNA并进行PCR扩增, 反应结束后, 以*U6*为内参, 采用$2^{-\Delta\Delta Ct}$方法计算miR-106b-5p相对表达量。miR-106b-5p上游引物5′→3′: GGG GCT AAA GTG CTG ACA GT, 下游引物5′→3′: GGA GCA GCA AGT ACC CAC AG; *U6*上游引物5′→3′: CTC GCT TCG GCA GCA CAT, 下游引物5′→3′: TTT GCG TGT CAT CCT TGC G。

1.2.4 CCK-8法检测细胞增殖 各组SCC15、OECM1细胞以每孔4×10^4个细胞接种至96孔板中培养, 在24 h、48 h、72 h时, 使用CCK-8试剂盒检测细胞增殖情况。

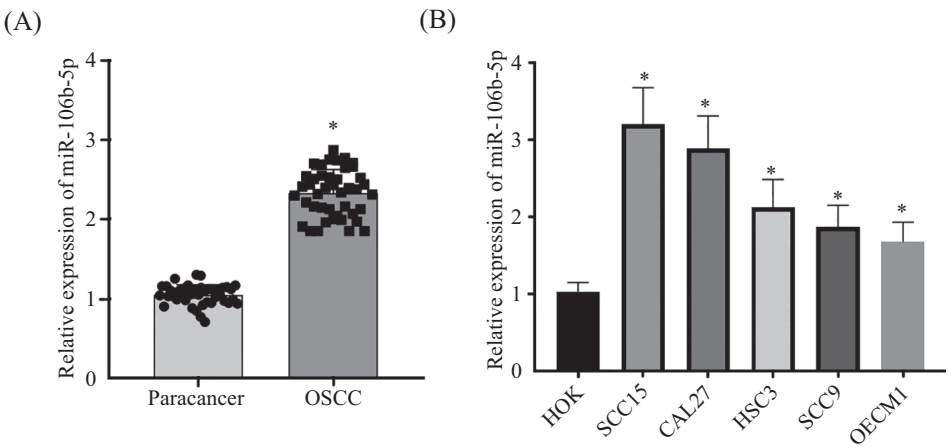
1.2.5 Transwell小室实验检测细胞迁移与侵袭 各组SCC15、OECM1细胞使用无血清培养基稀释, 接种至Transwell上室, 侵袭实验Transwell上室使用Matrigel预包被, Transwell下室加入完全培养基(含10%胎牛血清), 于37 ℃、5% CO_2的培养箱中培养2 h后, 室温下乙醇固定20 min, 并用结晶紫染色10 min。显微镜下观察并拍照, 随机选择5个视野计算穿膜细胞数。

1.2.6 Western blot检测SMAD4、SIRT7、E-cadherin、N-cadherin、MMP-9蛋白表达情况 用RIPA裂解液提取细胞蛋白, 蛋白定量后, 用SDS-PAGE分离等量蛋白样品并转膜, 将膜在室温下用5%脱脂牛奶封闭60 min。将膜与一抗(E-cadherin、N-cadherin、MMP-9、SIRT7、SMAD4及β-actin, 1:1 000稀释)在4 ℃下孵育过夜, 次日加入相应HRP标记的IgG二抗(1:4 000稀释)室温孵育2 h, ECL显影, 使用Image-Pro Plus 6软件观察并量化蛋白条带的灰度值。以β-actin为内参, 计算目的蛋白相对表达量。

1.2.7 双荧光素酶报告基因实验验证miR-106b-5p与*SIRT7*靶向关系 生物信息学网站(StarBase、targetscan)预测miR-106b-5p与*SIRT7* 3′-UTR的靶向结合位点。将miR-106b-5p结合的*SIRT7* 3′-UTR的野生型(wt)序列融合到pmirGLO载体中, 并将其命名为SIRT7-wt载体, 将miR-106b-5p靶向的*SIRT7* 3′-UTR序列进行定点突变, 将突变型(mut) SIRT7 3′-UTR克隆到pmirGLO载体中, 将其命名为SIRT7-mut。用Lipofectamine 2000将SIRT7-wt、SIRT7-mut分别与miR-NC、miR-106b-5p mimics共转染SCC15、OECM1细胞48 h, 检测相对荧光素酶活性。

1.2.8 RIP实验 将含有Ago2抗体或IgG抗体的磁珠与SCC15、OECM1细胞裂解液4 ℃下孵育过夜, 离心洗涤磁珠, 蛋白酶K消化后提取RNA, qRT-PCR检测miR-106b-5p表达。

1.2.9 统计学分析 采用SPSS 25.0软件统计分析, 实验数据以$\bar{x}\pm s$表示, 两组间比较用*t*检验, 多组间比较采用单因素方差分析和SNK-*q*检验。$P<0.05$表示



A: miR-106-5p在OSCC组织中的表达; B: miR-106-5p在OSCC细胞系中的表达。*$P<0.05$, 与Paracancer或HOK组比较。

A: expression of miR-106-5p in OSCC tissue; B: expression of miR-106-5p in OSCC cell line. *$P<0.05$ compared with Paracancer or HOK group.

图1 mR-106b-5p在OSCC组织与细胞系中的表达

Fig.1 Expression of mR-106b-5p in OSCC tissue and cell line

差异有统计学意义。

2 结果

2.1 miR-106b-5p在OSCC组织及细胞系中表达

miR-106b-5p在OSCC组织中表达水平高于癌旁组织[(2.36±0.52)比(1.05±0.12), t=16.097, P<0.05)](图1A); miR-106b-5p在OSCC细胞系SCC15、CAL27、HSC3、SCC9、OECM1中表达水平高于HOK细胞(t=11.008、10.430、7.100、6.754、5.742, P<0.05)(图1B)。此外, miR-106b-5p的相对表达量在OSCC组织不同TNM分期、分化程度和淋巴结转移方面, 差异有统计学意义(P<0.05)(表1)。

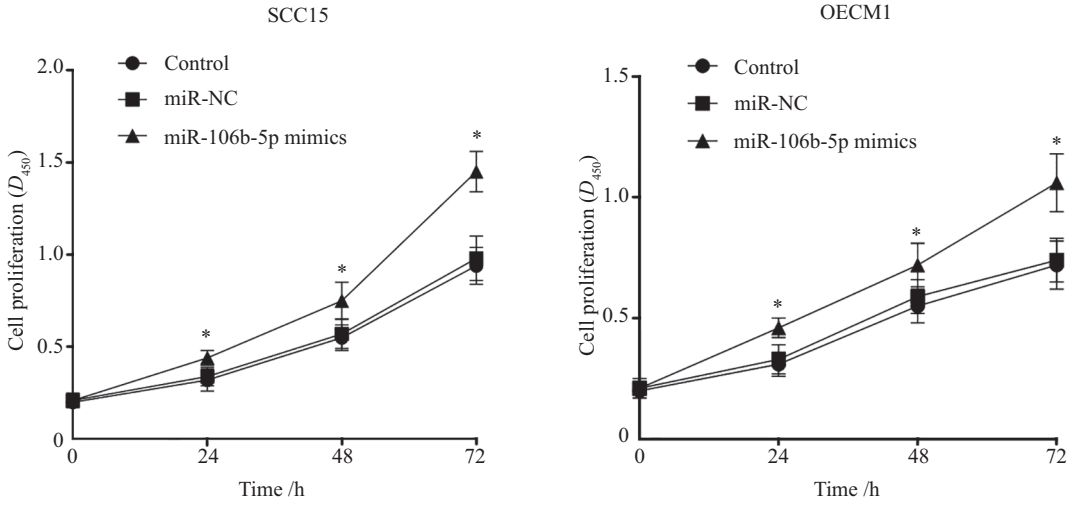
2.2 过表达miR-106b-5p对OSCC细胞的影响

与miR-NC组比较, miR-106b-5p mimics组细胞增殖活性、迁移与侵袭细胞数及N-cadherin、MMP-9表达水平升高, E-cadherin表达水平降低(P<0.05), Control组与miR-NC组各指标比较差异无统计学意义(P＞0.05)(图2~图4、表2、表3)。

表1 不同临床病理特征的OSCC组织中miR-106b-5p相对表达量比较

Table 1 Comparison of miR-106b-5p relative expression levels in OSCC tissues with different clinicopathological characteristics

临床特征 Clinical features	例数 Number of cases	miR-106b-5p相对表达量 Relative expression of miR-106b-5p	t	P
Gender			0.124	0.902
Male	25	2.35±0.49		
Female	18	2.37±0.56		
Age /year			0.250	0.804
＞50	21	2.34±0.46		
≤50	22	2.38±0.58		
TNM staging			2.326	0.025
I+II	20	2.16±0.47		
III+IV	23	2.53±0.56		
Degree of differentiation			2.312	0.026
Poorly differentiated	18	2.57±0.50		
Medium and high differentiation	25	2.20±0.53		
Lymph node metastasis			2.444	0.019
Yes	16	2.61±0.48		
No	27	2.21±0.54		



*P<0.05, 与miR-NC组比较。

*P<0.05 compared with miR-NC group.

图2 CCK-8法检测OSCC细胞增殖

Fig.2 CCK-8 method for detecting OSCC cell proliferation

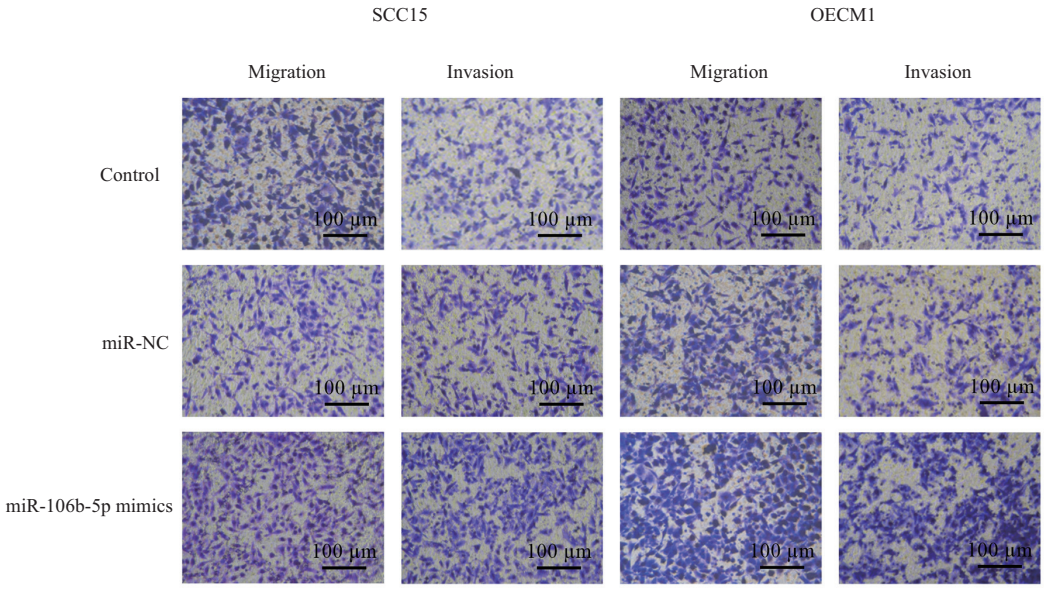


图3 Transwell检测细胞迁移与侵袭

Fig.3 Transwell detection of cell migration and invasion

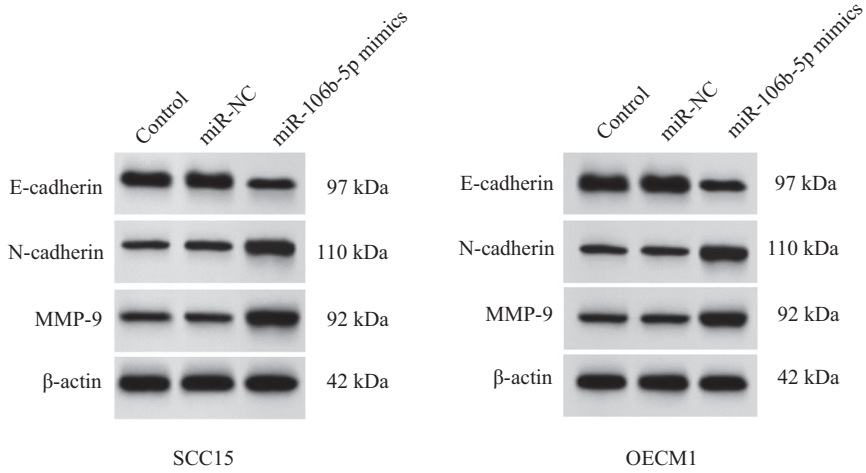


图4 Western blot检测E-cadherin、N-cadherin、MMP-9蛋白表达情况

Fig.4 Western blot detection of E-cadherin, N-cadherin, and MMP-9 protein expression

表2 过表达miR-106b-5p对SCC15细胞迁移、侵袭及EMT的影响

Table 2 Effects of overexpression of miR-106b-5p on migration, invasion and EMT of SCC15 cells

组别 Group	迁移细胞数 Number of migrating cells	侵袭细胞数 Number of invading cells	E-cadherin	N-cadherin	MMP-9
Control	135.27±8.31	126.53±7.23	0.85±0.10	0.51±0.07	0.31±0.04*
miR-NC	132.64±7.56	129.35±7.73	0.83±0.09	0.55±0.06	0.34±0.05*
miR-106b-5p mimics	178.33±8.68*	165.37±8.36*	0.21±0.04*	1.15±0.12*	0.97±0.10*

n=6。**P*<0.05, 与miR-NC组比较。

n=6. **P*<0.05 compared with miR-NC group.

2.3 抑制miR-106b-5p表达对OSCC细胞的影响

与anti-miR-NC组比较，anti-miR-106b-5p组细胞增殖活性、迁移与侵袭细胞数及N-cadherin、MMP-9表达水平降低，E-cadherin表达水平升高(*P*<0.05)(图5~图7、表4、表5)。

2.4 miR-106b-5p与SIRT7的靶向关系验证

经根据生物信息学分析显示，miR-106b-5p与*SIRT7* 3′-UTR有靶向结合位点(图8)，与SIRT7-

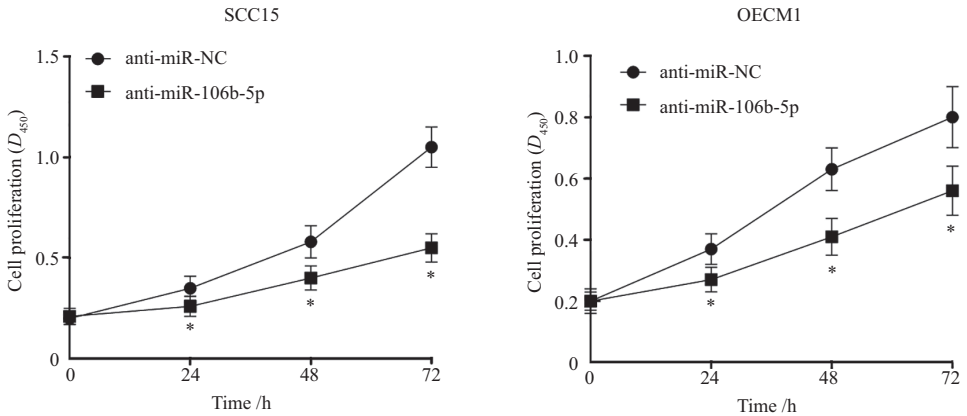
表3 过表达miR-106b-5p对OECM1细胞迁移、侵袭及EMT的影响

Table 3 Effects of overexpression of miR-106b-5p on migration, invasion and EMT of OECM1 cells

组别 Group	迁移细胞数 Number of migrating cells	侵袭细胞数 Number of invading cells	E-cadherin	N-cadherin	MMP-9
Control	136.42±7.69	125.61±6.87	0.83±0.08	0.54±0.06	0.37±0.05*
miR-NC	138.25±8.12	128.36±7.34	0.84±0.09	0.57±0.08	0.35±0.06*
miR-106b-5p mimics	182.64±9.34*	159.26±8.36*	0.25±0.04*	1.21±0.12*	1.01±0.12*

n=6。**P*<0.05, 与miR-NC组比较。

n=6. **P*<0.05 compared with miR-NC group.



**P*<0.05, 与anti-miR-NC组比较。

**P*<0.05 compared with anti-miR-NC group.

图5 CCK-8法检测OSCC细胞增殖

Fig.5 CCK-8 method for detecting OSCC cell proliferation

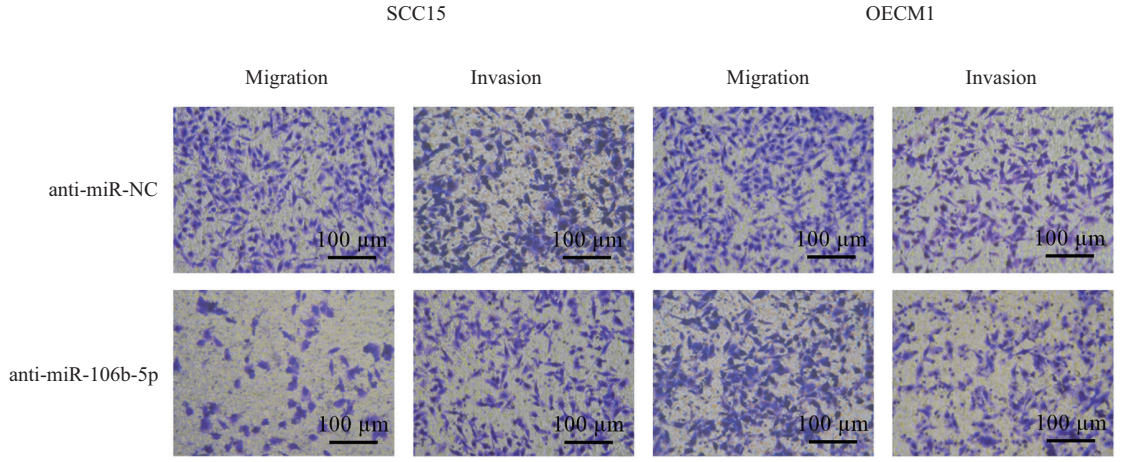


图6 Transwell检测细胞迁移与侵袭

Fig.6 Transwell detection of cell migration and invasion

wt+miR-NC组比较, SIRT7-wt+miR-106b-5p mimics组SCC15、OECM1细胞荧光素酶活性降低(*P*<0.05)(图9)。RIP结果显示, 与IgG组比较, Ago2组miR-106b-5p表达水平显著升高(*P*<0.05)(图10)。

2.5 各组miR-106b-5p与SIRT7、SMAD4表达

与miR-NC组比较, miR-106b-5p mimics组miR-106b-5p、SMAD4表达水平升高, SIRT7表达水平升高(*P*<0.05)(图11与表6); 与anti-miR-NC组比较, anti-miR-106b-5p组, miR-106b-5p、SMAD4表达水平降低, SIRT7表达水平升高(*P*<0.05)(图12与表7)。

2.6 下调SIRT7逆转抑制miR-106b-5p对OSCC细胞恶性生物学行为的影响

与anti-miR-106b-5p+si-NC组比较, anti-

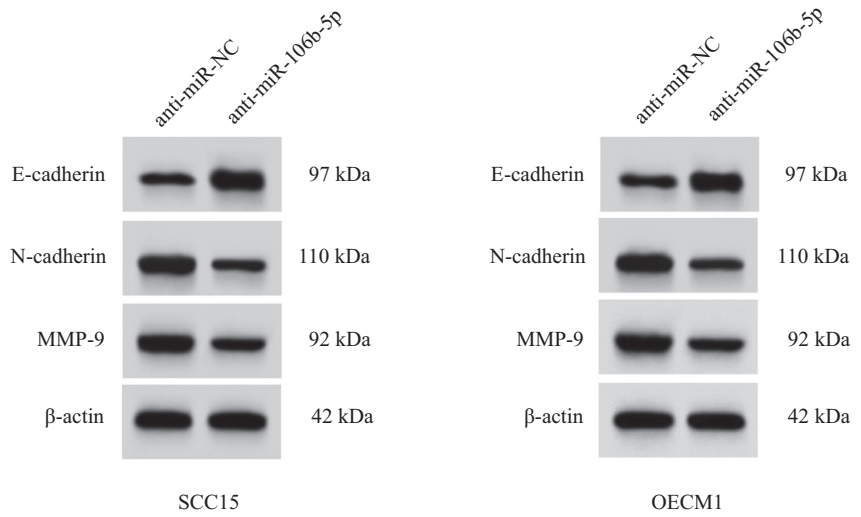


图7 Western blot检测E-cadherin、N-cadherin、MMP-9蛋白表达情况

Fig.7 Western blot detection of E-cadherin, N-cadherin, and MMP-9 protein expression

表4 抑制miR-106b-5p表达对SCC15细胞迁移、侵袭及EMT的影响

Table 4 Effects of inhibiting miR-106b-5p expression on migration, invasion and EMT of SCC15 cells

组别 Group	迁移细胞数 Number of migrating cells	侵袭细胞数 Number of invading cells	E-cadherin	N-cadherin	MMP-9
anti-miR-NC	141.36±8.24	124.62±7.55	0.21±0.04	1.13±0.12	0.98±0.10
anti-miR-106b-5p	68.33±4.68	55.47±4.25	0.87±0.08	0.51±0.07	0.43±0.06
t	18.877	19.550	18.075	10.932	11.552
P	<0.001	<0.001	<0.001	<0.001	<0.00

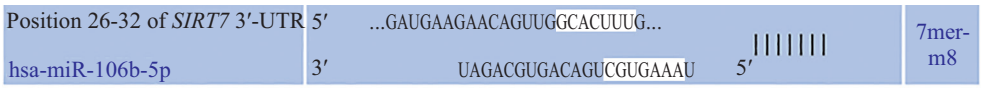
n=6.

表5 抑制miR-106b-5p表达对OECM1细胞迁移、侵袭及EMT的影响

Table 5 Effects of inhibiting miR-106b-5p expression on migration, invasion and EMT of OECM1 cells

组别 Group	迁移细胞数 Number of migrating cells	侵袭细胞数 Number of invading cells	E-cadherin	N-cadherin	MMP-9
anti-miR-NC	139.54±7.63	126.64±6.82	0.25±0.04	1.15±0.11	1.02±0.11
anti-miR-106b-5p	70.24±5.12	58.42±4.45	0.90±0.10	0.53±0.06	0.45±0.06
t	18.474	13.843	14.783	12.120	11.143
P	<0.001	<0.001	<0.001	<0.001	<0.00

n=6.



白色标记区域为预测的结合位点。

The white marked area is the predicted binding site.

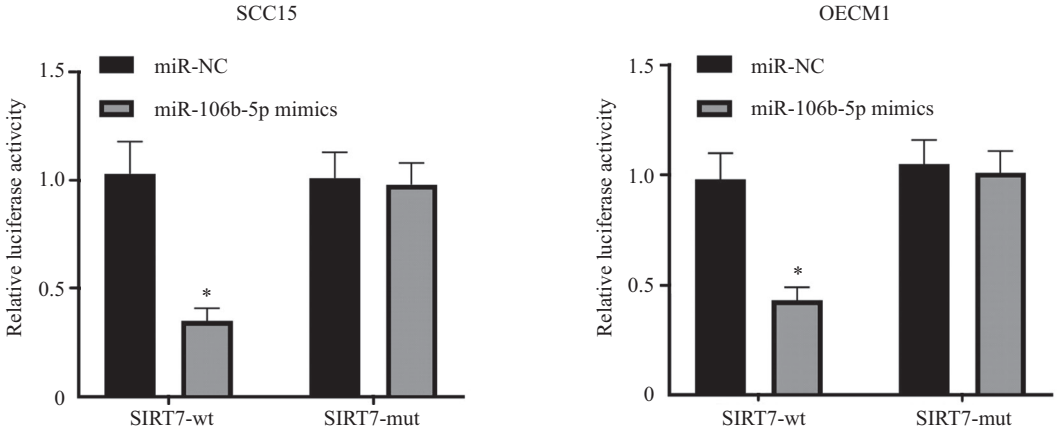
图8 miR-106b-5p与*SIRT7*结合位点预测

Fig.8 Prediction of binding sites between miR-106b-5p and *SIRT7*

miR-106b-5p+si-SIRT7组细胞增殖活性、迁移与侵袭细胞数及N-cadherin、MMP-9、SMAD4表达水平升高，miR-106b-5p、E-cadherin表达水平降低 *P*<0.05)(图13~图15、表8、表9)。

3 讨论

尽管OSCC的治疗方式(包括化疗、放疗和靶向治疗)取得了显著进步，但患者的生存率仍然较低[8]。因此，寻找新的治疗靶点对OSCC的治疗具有重要意

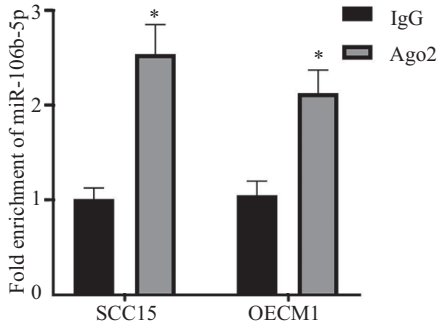


*P<0.05, 与miR-NC组比较。

*P<0.05 compared with miR-NC group.

图9 双荧光素酶活性检测结果

Fig.9 Test results of double luciferase activity



*P<0.05, 与IgG组比较。

*P<0.05 compared with IgG group.

图10 RIP实验结果

Fig.10 RIP experimental results

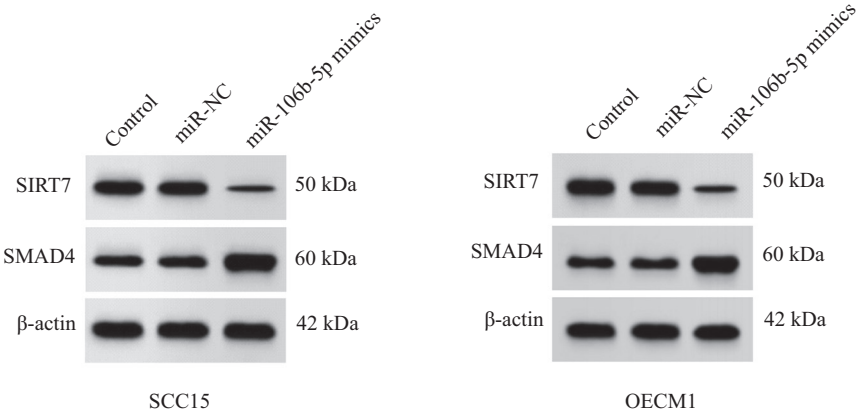


图11 Wetsern blot检测SIRT7蛋白表达情况

Fig.11 Wetsern blot detection of SIRT7 protein expression

义。据报道, miRNA与OSCC的恶性进展有关[9-11]。本研究从基因靶向治疗的角度出发, 探究miR-106b-5p对OSCC的影响及其作用机制。

miR-106b-5p在多数肿瘤中高表达, 发挥致癌基因作用, 如miR-106b-5p在乳腺癌、宫颈癌等肿瘤中表达水平升高, 与患者预后和肿瘤进展密切相关[12-13]。

表6 各组细胞miR-106b-5p、SIRT7、SMAD4蛋白表达情况

Table 6 Expression of miR-106b-5p, SIRT7, and SMAD4 proteins in each group of cells

组别	SCC9			OECM1		
Group	miR-106b-5p	SIRT7	SMAD4	miR-195-5p	SIRT7	SMAD4
Control	1.01±0.10	0.83±0.09	0.40±0.04	1.03±0.11	0.94±0.11	0.55±0.06
miR-NC	1.05±0.12	0.81±0.10	0.42±0.05	1.01±0.14	0.97±0.09	0.57±0.07
miR-106b-5p mimics	2.73±0.54*	0.21±0.04*	1.17±0.12*	2.61±0.42*	0.32±0.04*	1.21±0.13*
F	54.896	113.421	187.427	72.900	111.165	99.874
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

n=6。**P*<0.05, 与miR-NC组比较。

n=6. **P*<0.05 compared with miR-NC group.

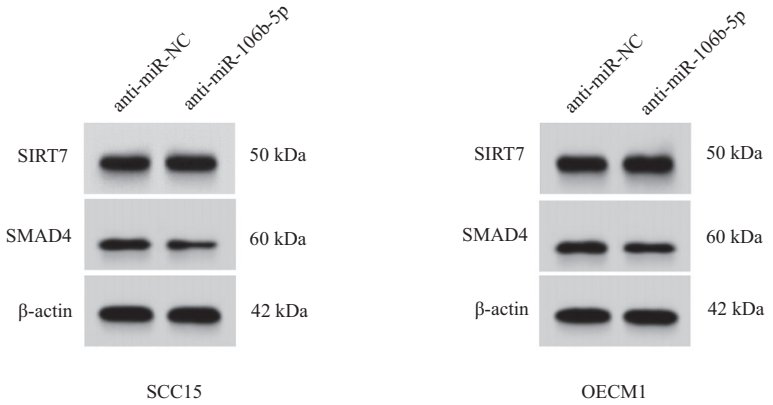


图12 Wetsern blot检测SIRT7、SMAD4蛋白表达水平

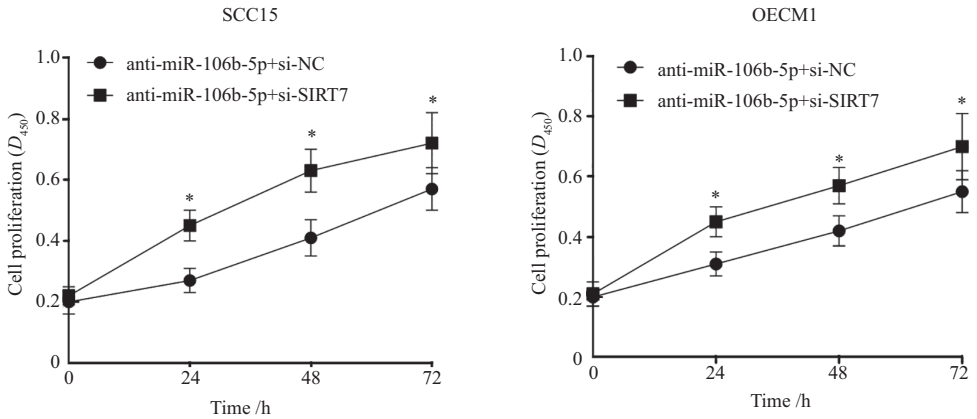
Fig.12 Wetsern blot detection of SIRT7 and SMAD4 protein expression

表7 各组细胞miR-106b-5p、SIRT7、SMAD4蛋白表达情况

Table 7 Expression of miR-106b-5p, SIRT7, and SMAD4 proteins in each group of cells

组别	SCC15			OECM1		
Group	miR-106b-5p	SIRT7	SMAD4	miR-195-5p	SIRT7	SMAD4
anti-miR-NC	1.01±0.13	0.85±0.10	0.45±0.06	1.03±0.15	0.95±0.10	0.56±0.08
anti-miR-106b-5p	0.41±0.06	1.07±0.13	0.19±0.03	0.31±0.04	1.19±0.14	0.33±0.04
t	10.265	3.286	9.494	11.361	3.417	6.299
P	<0.001	0.008	<0.001	<0.001	0.007	<0.001

n=6.



**P*<0.05, 与anti-miR-106b-5p+si-NC组比较。

**P*<0.05 compared with anti-miR-106b-5p+si-NC group.

图13 CCK-8法检测OSCC细胞增殖

Fig.13 CCK-8 method for detecting OSCC cell proliferation

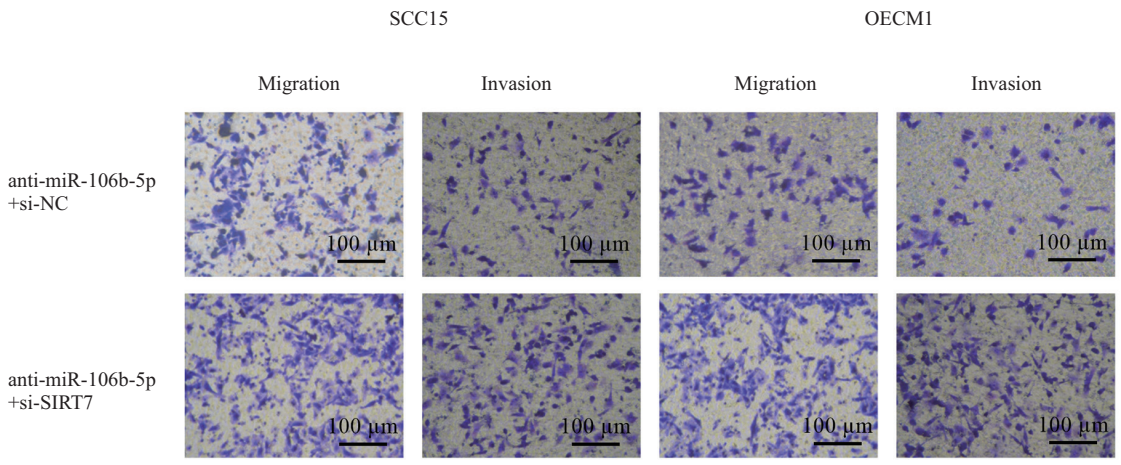


图14 Transwell检测细胞迁移与侵袭

Fig.14 Transwell detection of cell migration and invasion

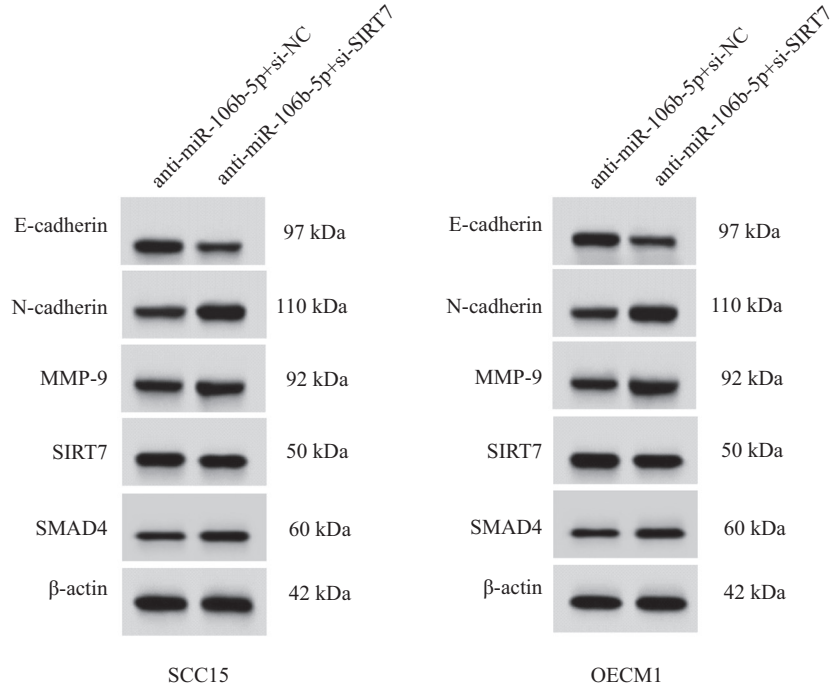


图15 Western blot检测蛋白表达水平

Fig.15 Western blot detection of protein expression

表8 同时抑制SIRT7与miR-106b-5p表达对SCC15细胞迁移、侵袭及EMT的影响

Table 8 Effects of simultaneous inhibition of SIRT7 and miR-106b-5p expression on migration, invasion and EMT of SCC15 cells

组别 Group	迁移细胞数 Number of migrating cells	侵袭细胞数 Number of invading cells	E-cadherin	N-cadherin	MMP-9	SIRT7	SMAD4
anti-miR-106b-5p+si-NC	70.25±5.72	56.32±4.84	0.85±0.07	0.52±0.06	0.45±0.05	1.18±0.12	0.33±0.04
anti-miR-106b-5p+si-SIRT7	128.45±6.74	117.21±6.25	0.30±0.05	1.08±0.11	0.84±0.10	1.01±0.10	0.45±0.06
t	16.127	18.868	15.661	10.947	8.544	2.666	4.046
P	<0.001	<0.001	<0.001	<0.001	<0.00	0.024	0.002

n=6.

表9 同时抑制SIRT7与miR-106b-5p表达对OECM1细胞迁移、侵袭及EMT的影响

Table 9 Effects of simultaneous inhibition of SIRT7 and miR-106b-5p expression on migration, invasion and EMT of OECM1 cells

组别 Group	迁移细胞数 Number of migrating cells	侵袭细胞数 Number of invading cells	E-cadherin	N-cadherin	MMP-9	SIRT7	SMAD4
anti-miR-106b-5p+si-NC	68.27±5.22	55.41±5.31	0.87±0.09	0.53±0.05	0.43±0.04	1.15±0.12	0.31±0.03
anti-miR-106b-5p+si- SIRT7	125.64±7.24	112.58±6.15	0.35±0.05	1.05±0.11	0.89±0.08	0.98±0.11	0.47±0.05
t	15.744	17.235	12.372	10.542	12.598	2.558	6.721
P	<0.001	<0.001	<0.001	<0.001	<0.001	0.028	<0.001

n=6.

本研究发现miR-106b-5p在OSCC组织与细胞系中高表达，提示miR-106b-5p可能与OSCC的发生有关。为了明确miR-106b-5p在OSCC中的功能，本研究在SCC15、OECM1细胞中过表达miR-106b-5p，发现miR-106b-5p可促进细胞增殖、迁移和侵袭，而抑制miR-106b-5p表达则发挥相反作用，表明miR-106b-5p促进OSCC进展。转移是影响肿瘤治疗的障碍之一，EMT是OSCC恶性进展与转移的重要过程，EMT的激活导致上皮标志物E-cadherin表达水平降低，黏附和间充质蛋白N-cadherin表达水平增加，MMP-9可促进肿瘤细胞迁移与侵袭[14-15]。本研究显示，抑制miR-106b-5p表达后，N-cadherin、MMP-9表达水平降低，E-cadherin表达水平升高，提示下调miR-106b-5p通过抑制EMT抑制OSCC细胞的迁移和侵袭。

miR-106b-5p通过靶向调控多种基因表达，参与调控肿瘤细胞增殖、迁移与侵袭，且miR-106b-5p与肿瘤恶性进展及耐药性有关[16]。例如，在乳腺癌中，miR-106b-5p可通过抑制CNN1和激活Rho/ROCK1通路促进乳腺癌细胞增殖、迁移、侵袭及肺转移[17]。在食管癌中，下调miR-106b-5p表达可抑制食管癌恶性进展[18]。此外，miR-106b-5p可通过下调IGSF10抑制肺腺癌细胞的生长和进展[19]。本研究经双荧光素酶报告基因实验与RIP实验证实，miR-106b-5p与*SIRT7*存在靶向关系，且在OSCC细胞中过表达miR-106b-5p或抑制miR-106b-5p表达可抑制或上调SIRT7表达，下调SIRT7表达可逆转抑制miR-106b-5p表达对OSCC细胞恶性表型的抑制作用，提示miR-106b-5p可能通过靶向调节SIRT7表达参与调控OSCC细胞增殖、迁移与侵袭。

SIRT7参与上皮细胞向间质细胞转化过程抑制肿瘤进展[20-21]。SMAD4是SMAD信号转导的重要因子，在肿瘤转移过程中发挥重要作用[22]。研究显示，在OSCC中，SIRT7通过促进SMAD4去乙酰化来抑制EMT在OSCC转移中的作用[23]。本研究发现，在OSCC细胞中过表达miR-106b-5p或抑制miR-106b-5p表达，SMAD4表达水平升高或降低，抑制miR-106b-5p表达的同时抑制SIRT7表达，SMAD4表达水平升高，表明下调miR-106b-5p表达可通过靶向抑制SIRT7/SMAD4通路，抑制OSCC细胞增殖、迁移、侵袭及EMT。本研究仅探索miR-106b-5p在OSCC细胞中的作用，未进行动物体内实验为本研究不足之处，后续将结合动物模型作进一步研究。

综上所述，miR-106b-5p在OSCC组织与细胞中表达上调，抑制miR-106b-5p表达可通过调节SIRT7/SMAD4信号通路，抑制OSCC细胞增殖、迁移、侵袭及EMT。

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