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· 临床研究 ·

## lncRNA XIST/miR-34a-5p/SIRT6 分子轴调控口腔鳞癌细胞的增殖及转移

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**[摘要]** **目的:** 探究 lncRNA XIST/miR-34a-5p/SIRT6 分子轴调控口腔鳞癌细胞增殖和转移及其分子机制。**方法:** 收集 2013 年 3 月至 2018 年 3 月在青岛市口腔医院就诊的 OSCC 患者 47 例癌组织和癌旁组织标本, 采用 qPCR 检测 OSCC 患者组织及细胞系中 lncRNA XIST、miR-34a-5p、SIRT6 mRNA 的表达, WB 检测 OSCC 患者组织及细胞系中 SIRT6、Ki67、pcDNA、cleaved-caspase3、cleaved-caspase8、E-cadherin、Vimentin 蛋白的表达, 采用 CCK-8 实验检测敲降 lncRNA XIST 对 Cal-27 及 Tca-8113 细胞增殖的影响, Transwell 小室法检测 Cal-27 及 Tca-8113 细胞迁移及侵袭; 流式细胞术检测 Cal-27 及 Tca-8113 细胞凋亡情况, 双荧光素酶报告基因检测 lncRNA XIST 与 miR-34a-5p、miR-34a-5p 与 SIRT6 靶向结合关系。**结果:** lncRNA XIST 和 SIRT6 在 OSCC 患者癌组织及细胞系中高表达(均  $P < 0.05$ ), miR-34a-5p 则呈低表达( $P < 0.01$ ); 敲降 lncRNA XIST 抑制 OSCC 细胞的增殖、迁移及侵袭并促进细胞凋亡(均  $P < 0.01$ ), 同时转染 miR-34a-5p 抑制剂或 pcDNA-SIRT6 载体作用则相反; 敲降 lncRNA XIST 促进 OSCC 细胞中增殖及转移相关蛋白表达(均  $P < 0.01$ ), 同时转染 miR-34a-5p 抑制剂或 pcDNA-SIRT6 载体作用则相反; lncRNA XIST 与 miR-34a 靶向结合, miR-34a 与 SIRT6 靶向结合; lncRNA XIST 通过靶向 miR-34a-5p 上调 SIRT6 表达( $P < 0.01$ )。**结论:** lncRNA XIST/miR-34a-5p/SIRT6 分子轴能够调控 OSCC 细胞增殖及转移, 为 OSCC 治疗提供潜在靶点。

**[关键词]** 长链非编码 RNA XIST; miR-34a-5p; SIRT6; 口腔鳞癌; Cal-27 细胞; Tca-8113 细胞; 增殖; 转移

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## lncRNA XIST/miR-34a-5p/SIRT6 axis regulates proliferation and metastasis of oral squamous cell carcinoma cells

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**[Abstract]** **Objective:** To investigate the molecular mechanisms of lncRNA XIST/miR-34a-5p/SIRT6 axis regulating the proliferation and metastasis of oral squamous cell carcinoma (OSCC) cells. **Methods:** Specimens of tumor tissues and paracancer tissues of 47 patients with OSCC, who visited the Qingdao Stomatological Hospital from March 2013 to March 2018, were enrolled in this study. qPCR was performed to measure the mRNA expressions of lncRNA XIST, miR-34a-5p and SIRT6 in OSCC tissues and cell lines. WB was performed to measure the protein levels of SIRT6, Ki67, pcDNA, cleaved-caspase3, cleaved-caspase8, E-cadherin and vimentin in OSCC tissues and cell lines. CCK-8 assay was performed to observe the effect of lncRNA XIST knockdown on proliferation of Cal-27 and Tca-8113 cells; Tanswell assay was performed to detect migration and invasion of Cal-27 and Tca-8113 cells; flow cytometry was performed to detect the apoptosis of Cal-27 and Tca-8113 cells; and dual luciferase reporter assay was performed to verify the relationship between lncRNA XIST and miR-34a, or miR-34a and SIRT6. **Results:** Expressions of lncRNA XIST and SIRT6 were up-regulated in OSCC tissues and cell lines (all  $P < 0.05$ ), reversely, miR-34a-5p was down-regulated in OSCC tissues and cell lines ( $P < 0.01$ ). lncRNA XIST knockdown significantly suppressed OSCC cells proliferation, migration and invasion, and induced apoptosis of OSCC cells (all  $P < 0.01$ ); however, simultaneous transfection with miR-34a-5p inhibitor or pcDNA-SIRT6 vector exerted opposite effect. lncRNA XIST knockdown significantly increased cell proliferation and metastasis related protein expression in OSCC cells (all  $P < 0.01$ ), and simultaneous transfection with miR-34a-5p inhibitor or pcDNA-SIRT6 vector exerted opposite effect. In addition, lncRNA XIST directly targeted miR-34a-5p, and miR-34a-5p targeted SIRT6, lncRNA XIST upregulated SIRT6 expression via inhibiting miR-34a-5p ( $P < 0.01$ ). **Conclusion:** Taken together, lncRNA XIST/miR-34a-5p/SIRT6 axis regulates the proliferation and metastasis of OSCC cells and provides potential therapeutic targets for OSCC.

**[Key words]** lncRNA XIST; miR-34a-5p; SIRT6; oral squamous cell carcinoma (OSCC); Cal-27 cell; Tca-8113 cell; proliferation; metastasis

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口腔鳞状细胞癌(oral squamous cell carcinoma, OSCC; 简称口腔鳞癌)是最常见的头颈部肿瘤,约占头颈部肿瘤的90%<sup>[1]</sup>。OSCC的主要特点是局部浸润和颈部转移率高,导致OSCC术后容易复发,直接影响患者预后<sup>[2]</sup>。尽管近年来OSCC的诊断及治疗取得了巨大进展,但OSCC患者的预后水平仍未改善<sup>[3]</sup>,因此需要寻找更多OSCC转移相关的标志物来识别预后较差及易复发患者,为OSCC治疗提供新的参考靶点。lncRNA可通过调控miRNA的表达在肿瘤发生发展过程中发挥癌基因及抑癌基因作用,如lncRNA AF147447可通过上调miR-34c及靶基因MUC2抑制胃癌细胞的增殖及侵袭<sup>[4]</sup>,lncRNA HULC可通过调控mir-186/HMGA2分子轴促进肝癌的发展<sup>[5]</sup>,lncRNA RP4通过与miR-7-5p内源性竞争促进结直肠癌的发展进程<sup>[6]</sup>。

去乙酰化酶 sirtuin 6(SIRT6)是多种恶性肿瘤及其他某些疾病发展的关键调节因子<sup>[7]</sup>。Sirtuins属于NAD<sup>+</sup>依赖性去乙酰化酶家族,能够介导多种生物过程和各种疾病的发展,包括大多数肿瘤的发生发展<sup>[8-9]</sup>。SIRT6首次从人脾脏cDNA文库中克隆,SIRT6通过在特定组蛋白H3位点去乙酰化,抑制靶基因的转录<sup>[10]</sup>。此外,SIRT6控制或介导多种生物学功能,如染色体完整性的维持和炎症反应<sup>[11-12]</sup>,SIRT6还可能是多种实体瘤治疗的靶点<sup>[13]</sup>。SIRT6与CDC25A相互作用抑制结直肠癌干细胞的增殖<sup>[14]</sup>,SIRT6可通过与miR-34c-5p相互作用激活JAK2/STAT3信号通路促进结肠癌细胞增殖<sup>[15]</sup>。SIRT6影响肺癌恶性生物学行为,包括细胞分化、肿瘤和肿瘤TNM分期等<sup>[16-17]</sup>,在黑色素瘤和成神经细胞瘤中发挥癌基因作用<sup>[18-19]</sup>。然而,SIRT6在OSCC患者中的表达及其与OSCC临床特征的相关性尚未完全阐明,其在OSCC中的作用机制及其失调机制亦尚未被揭示。

## 1 材料与方法

### 1.1 临床样本收集

收集2013年3月至2018年3月在青岛市口腔医院就诊的47例OSCC患者的癌组织和癌旁组织标本,患者均未接受过化疗或者放疗,年龄35~56岁,其中男性24例、女性23例。本研究经过医院伦理委员会审查同意,患者签署知情同意书。

### 1.2 细胞株及主要试剂

人正常口腔角质化细胞hNOK及OSCC细胞系SCC-9、SCC-25、HN4、Tca-8113、Cal-27均购于中国科学院上海细胞库,其中hNOK作为对照组;细胞培养于含10%胎牛血清及100 U/ml青霉素、100 mg/ml链

霉素的DMEM培养基中。TRIzol购买于Invitrogen公司,High Capacity cDNA Reverse Transcription Kit购自Applied Biosystems公司,SYBR Premix Ex Taq试剂盒从TaKaRa公司购买,TaqMan MicroRNA Reverse Transcription Kit和TaqMan Universal Master Mix II试剂盒从Applied Biosystems公司购买,RAPI蛋白裂解液及BCA试剂盒购自Invitrogen公司,抗SIRT6、Ki67、pcDNA、cleaved-caspase3、cleaved-caspase8、E-cadherin、Vimentin、GAPDH等抗体均购于Abcam公司,羊抗兔IgG二抗从Invitrogen公司购买,ECL化学发光液购从BioRad公司购买,CCK-8试剂及Annexin V-FITC/PI细胞凋亡检测试剂盒购买于索莱宝公司,Lipofectamine 2000购买于Invitrogen公司,Dual-Luciferase Reporter Assay Kit购买于Promega公司,RT-PCR引物由上海生工设计并合成,lncRNA XIST-shRNA及阴性对照从Santa Cruz Biotechnology公司购买,miR-34a-5p模拟物、抑制剂及相应的阴性对照寡核苷酸、pcDNA3.1(+)-vector及pcDNA3.1-SIRT6 vector由上海吉玛基因设计并合成。

### 1.3 qPCR检测基因表达水平

使用TRIzol reagent试剂提取组织或细胞中总RNA,NanoDrop检测RNA的浓度及纯度,以RNA为模板进行逆转录合成cDNA,RT-PCR反应条件为37℃、1 h,70℃、15 min,合成的cDNA立即用于后续实验或于-20℃储存。以合成的cDNA为模板,分别按照TaqMan Universal Master Mix II试剂盒及SYBR Premix Ex Taq试剂盒及说明对miR-34a-5p及lncRNA XIST表达水平进行qPCR检测,分别以U6及GAPDH作为内参对照进行实验,qPCR反应条件为95℃、30 s,95℃、15 s,58℃、30 s,72℃、30 s,进行35个循环;72℃、7 min。实验设置3个重复,根据每孔荧光信号达到阈值时所经历的循环数为Ct值,以2<sup>-ΔΔCt</sup>法计算基因的相对表达量。

### 1.4 WB检测蛋白表达水平

使用RAPI裂解液提取细胞总蛋白,BCA试剂盒检测蛋白浓度及纯度,10% SDS-PAGE分离蛋白,采用半干转移法将蛋白转至PVDF膜上,5%脱脂奶粉室温封闭2 h后,加入按一定比例稀释的一抗(SIRT6,1:1 000;Ki67,1:2 000;pcDNA,1:1 000;cleaved-caspase3,1:2 000;cleaved-caspase8,1:1 000;E-cadherin,1:2 000;Vimentin,1:2 000;GAPDH,1:2 000)4℃过夜孵育,次日,去除一抗,TBST清洗3次后,加入相应的二抗,室温封闭1 h,最后加入ECL化学发光液置于凝胶成像系统中采集图像,实验设置3个重复,用Image J对蛋白条带进行半定量分析。

### 1.5 载体构建及细胞转染

转染前一天将细胞种于6孔板,使用lncRNA XIST-shRNA、对照shRNA、miR-34a-5p模拟物/抑制剂、阴性对照组寡核苷酸、pcDNA3.1-SIRT6 vector及pcDNA3.1(+)-vector分别与终浓度为50 nmol/L的Lipofectamine 2000脂质体混合转染Cal-27及Tca-8113细胞48 h,然后进行后续实验检测。

### 1.6 载体构建及双荧光素酶报告基因检测

使用PCR扩增包含miR-34a 3'UTR区结合位点的lncRNA XIST及SIRT6序列片段,并导入pmir-GLO luciferase表达载体中,获得lncRNA XIST及SIRT6野生型载体(pmirtGLO-XIST/SIRT6-WT),将lncRNA XIST序列ACACUGCC突变为AGUGACGC,将SIRT6序列CACUGCC突变为CUGACGC,分别获得lncRNA XIST及SIRT6突变型载体(pmirtGLO-XIST/SIRT6-MUT),将pmirGLO、lncRNA XIST/SIRT6野生型载体、lncRNA XIST/SIRT6突变型载体、miR-34a-5p模拟物及阴性对照组分别与Lipofectamine 2000脂质体混合后转染Cal-27及Tca-8113细胞,转染48 h后,根据双荧光素酶报告基因检测试剂盒说明检测荧光素酶活性。

### 1.7 CCK-8检测细胞增殖情况

实验前一天,按照 $2 \times 10^4$ 个/孔的细胞量将细胞种于96孔板中,每孔约100  $\mu$ l,置于37  $^{\circ}$ C培养箱中培养4~6 h至细胞完全贴壁,每孔加入10  $\mu$ l 10% CCK-8溶液,37  $^{\circ}$ C培养箱孵育2 h,然后使用酶标仪检测450 nm处的光密度(D)值,每组3个重复孔。

### 1.8 Annexin V-FITC/PI双染法检测细胞凋亡情况

使用不含EDTA的胰酶消化细胞,使用预冷的1 $\times$ PBS清洗细胞,加入300  $\mu$ l的1 $\times$ Binding Buffer和5  $\mu$ l的Annexin V-FITC混匀,避光、室温孵育15 min,上

机前5 min加入5  $\mu$ l的PI进行染色,上机前补加200  $\mu$ l的1 $\times$ Binding Buffer,使用流式细胞仪进行检测,每管样品重复检测3~5次。

### 1.9 Transwell检测细胞迁移及侵袭情况

侵袭实验开始前,使用孔径为8  $\mu$ m的聚碳酸酯膜Transwell小室进行实验,用50 mg/L Matrigel 1:8稀释包被Transwell小室底部膜的上室,37  $^{\circ}$ C放置30 min。实验前细胞无血清饥饿培养12~24 h,使用胰酶消化细胞,PBS清洗1~2次,用含BSA的无血清培养基重悬,调整细胞密度为 $4 \times 10^5$ 个细胞/100  $\mu$ l。接种细胞:取100  $\mu$ l细胞悬液加入Transwell小室,下室加入含20% FBS和DMEM/F12培养基。细胞常规培养于37  $^{\circ}$ C、5%CO<sub>2</sub>培养箱12 h。培养结束后,用棉签擦去基质胶和上室内细胞,用1%结晶紫染色20 min,并在显微镜下取3~5个视野进行计数。

进行细胞迁移实验不需要提前使用Matrigel胶包被Transwell小室,其余步骤与侵袭实验相同。

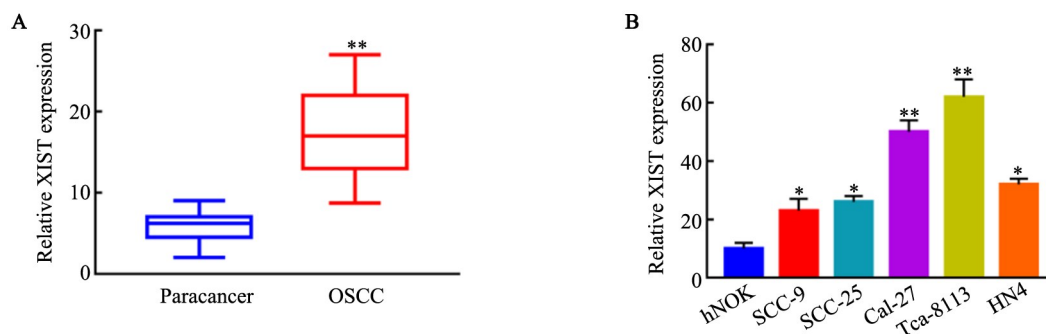
### 1.10 统计学处理

采用SPSS19.0进行数据统计,数据以 $\bar{x} \pm s$ 表示,两组间比较采用Student's t检验,多组间比较采用单因素ANOVA进行检测,以 $P < 0.05$ 或 $P < 0.01$ 表示差异具有统计学意义。

## 2 结果

### 2.1 lncRNA XIST在OSCC患者癌组织及细胞系中高表达

qPCR检测结果显示,与癌旁组织相比,lncRNA XIST在OSCC癌组织中表达明显增加( $t = -13.62$ ,  $P < 0.01$ ,图1A);在5种OSCC细胞系中lncRNA XIST的表达水平也明显高于人口腔正常角质化细胞hNOK ( $P < 0.05$ 或 $P < 0.01$ ,图1B)。



\* $P < 0.05$ , \*\* $P < 0.01$  vs paracancer group or hNOK cells group

A: Relative expressions of lncRNA XIST in OSCC tissues; B: Relative expressions of lncRNA XIST in OSCC cell lines

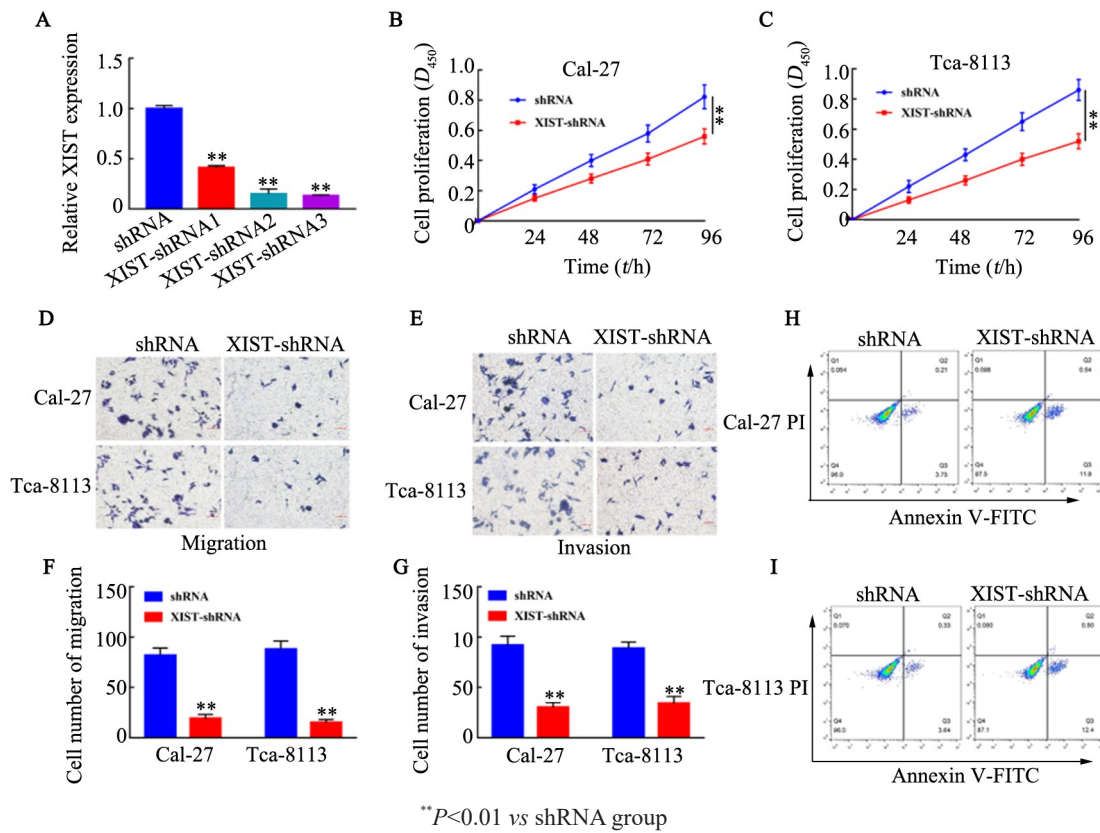
图1 lncRNA XIST在OSCC患者癌组织及细胞系中高表达

Fig.1 Expression of lncRNA XIST was up-regulated in OSCC tissues and cell lines

2.2 敲降 lncRNA XIST 抑制 OSCC 细胞的增殖、迁移及侵袭并促进细胞凋亡

敲降 OSCC 细胞中 lncRNA XIST 的表达后 ( $P < 0.01$ , 图 2A), 通过 CCK-8 及 Transwell 实验 lncRNA XIST 对 OSCC 细胞生物学的影响。结果显示, 敲降

lncRNA XIST 后, 显著抑制了 Cal-27 及 Tca-8113 细胞的增殖 ( $t=1.51、2.32$ , 均  $P < 0.01$ , 图 2B、C)、迁移 ( $t=89.10、103.24$ , 均  $P < 0.01$ , 图 2D、F) 及侵袭 ( $t=74.71、67.36$ , 均  $P < 0.01$ , 图 2E 和 G), 并促进了细胞凋亡 ( $t=-1041.03、-252.60$ , 均  $P < 0.01$ , 图 2H-I)。



A: Transfection of shRNA reduced expression of lncRNA XIST in OSCC cells; B, C: Knockdown of lncRNA XIST significantly inhibited the proliferation of Cal-27 and Tca-8113 cells; D, F: Knockdown of lncRNA XIST significantly inhibited the migration of Cal-27 and Tca-8113 cells ( $\times 100$ ); E, G: Knockdown of lncRNA XIST significantly inhibited the invasion of Cal-27 and Tca-8113 cells ( $\times 100$ ); H, I: Knockdown of lncRNA XIST significantly promoted apoptosis in Cal-27 and Tca-8113 cells

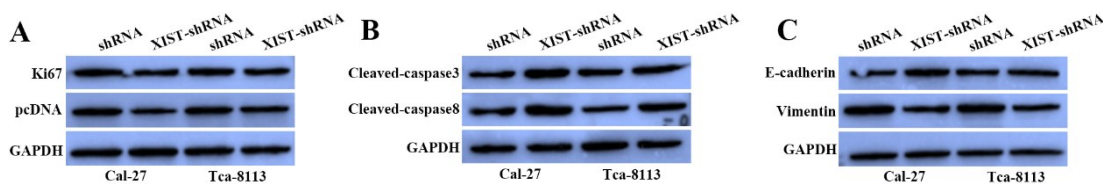
图2 敲降 lncRNA XIST 抑制 OSCC 细胞的增殖、迁移及侵袭并促进细胞凋亡

Fig.2 Knockdown of lncRNA XIST suppressed proliferation, migration and invasion and induced apoptosis of OSCC cells

2.3 敲降 lncRNA XIST 促进 OSCC 细胞增殖及转移相关蛋白表达

WB 实验检测结果显示, 敲降 lncRNA XIST 后显著抑制了两种 OSCC 细胞中 Ki67 ( $t=32.07、22.15$ )、pcDNA ( $t=54.71、37.26$ )、Vimentin ( $t=30.20、24.36$ ) 蛋

白的表达水平 (均  $P < 0.01$ , 图 3A、C), 上调了 E-cadherin ( $t=-52.36、-43.56$ )、cleaved-caspase3 ( $t=-74.56、-66.45$ )、cleaved-caspase8 ( $t=-94.61、-58.25$ ) 蛋白的表达水平 (均  $P < 0.01$ , 图 3B、C)。



A: Expression of Ki67 and pcDNA in Cal-27 and Tca-8113 cells by WB assay; B: Expression of cleaved-caspase3 and cleaved-caspase8 in Cal-27 and Tca-8113 cells by WB assay; C: Expression of E-cadherin and Vimentin in Cal-27 and Tca-8113 cells by WB assay

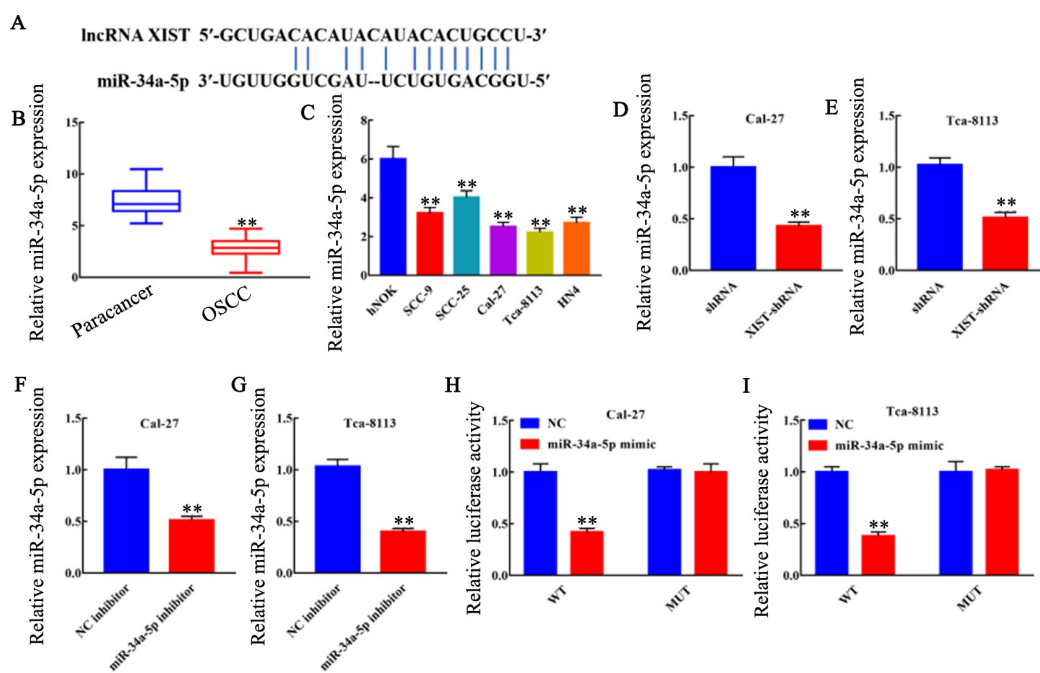
图3 敲降 lncRNA XIST 促进 OSCC 细胞增殖及转移相关蛋白表达

Fig.3 Knockdown of lncRNA XIST promoted OSCC cell proliferation and metastasis-related proteins

### 2.4 lncRNA XIST 与 miR-34a 靶向结合

通过 Starbase 软件预测发现, lncRNA XIST 与 miR-34a-5p 可直接靶向结合(图 4A), 通过 qPCR 检测发现, 与癌旁组织相比, miR-34a-5p 在 OSCC 患者癌组织中表达明显下调( $t=1.66, P<0.01$ , 图 4B); 且在人口腔正常角质化细胞 hNOK 中的表达明显高于 OSCC 细胞系(均  $P<0.01$ , 图 4C); 敲降 lncRNA XIST 明显抑制了 miR-34a-5p 在 Cal-27 及 Tca-8113 细胞中的表达( $t=54.71, 48.07$ , 均  $P<0.01$ , 图 4D、E), 与 miR-34a

抑制剂转染细胞的结果一致( $t=37.75, 60.40$ , 均  $P<0.01$ , 图 4F、G)。采用双荧光素酶报告基因检测 pmirGLO-XIST WT/MUT 与 miR-34a-5p 模拟物后转染细胞后的荧光素酶活性, 结果显示, 转染 pmirGLO-XIST WT 及 miR-34a-5p 模拟物后显著抑制了 Cal-27 及 Tca-8113 细胞的荧光素酶活性( $t=39.69, 59.45$ , 均  $P<0.01$ , 图 4H、I), 而转染 pmirGLO-XIST MUT 及 miR-34a-5p 模拟物后对细胞的荧光素酶活性无影响( $t=-0.40, -1.26, P>0.05$ , 图 4H、I)。



\*\* $P<0.01$  vs Paracancer group, or hNOK cells group, or shRNA group, or NC inhibitor group, or NC group

A: Starbase software predicted that lncRNA XIST and miR-34a-5p can be directly targeted to bind;

B: The expression of miR-34a-5p in OSCC tissues was lower than that in adjacent normal tissues;

C: Expression of miR-34a-5p in human oral normal keratinocytes cell hNOK was significantly higher than that of OSCC cell lines;

D, E: Knockdown of lncRNA XIST significantly inhibited the expression of miR-34a-5p in Cal-27 and Tca-8113 cells;

F, G: Transfection of miR-34a inhibitor inhibited the expression of miR-34a-5p in Cal-27 and Tca-8113 cells;

H, I: Transfection of pmirGLO-XIST WT and miR-34a-5p mimics inhibited luciferase activity in Cal-27 and Tca-8113 cells

图 4 lncRNA XIST 与 miR-34a-5p 靶向结合并调控其表达

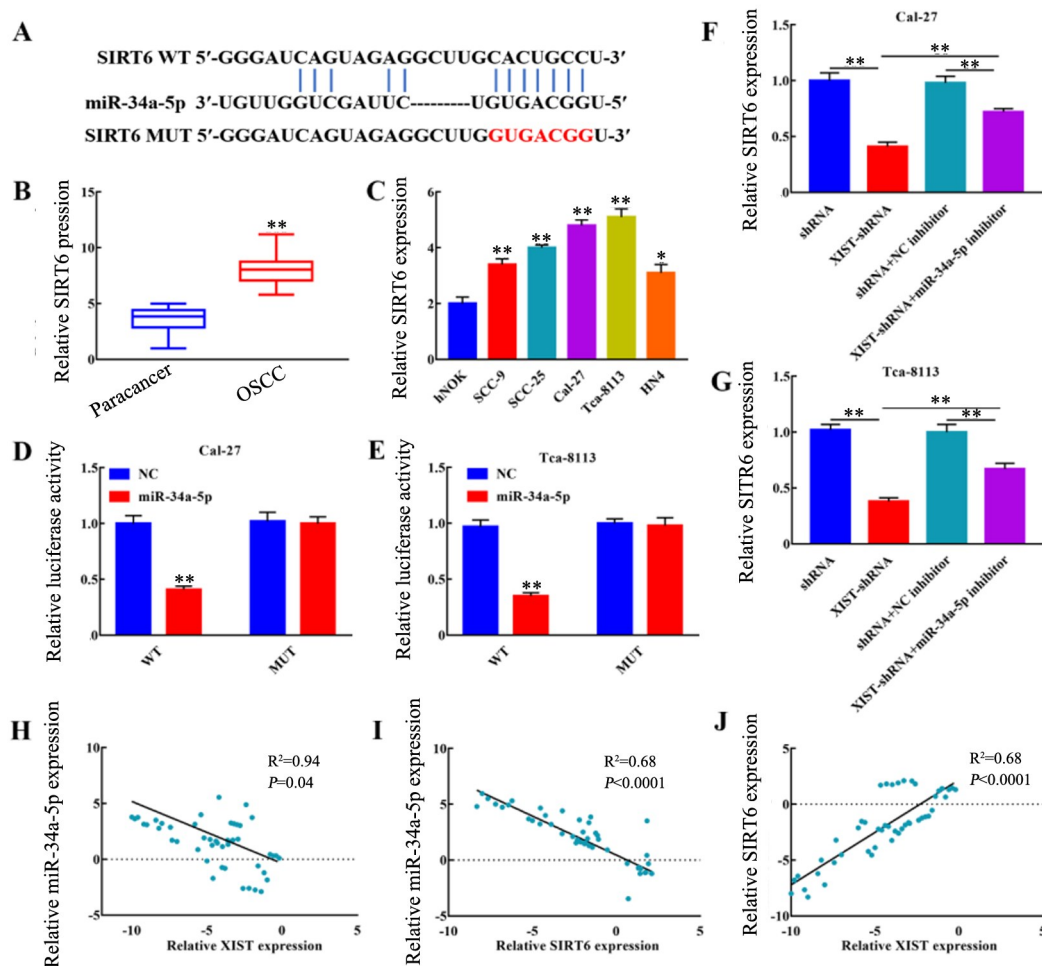
Fig.4 lncRNA XIST directly targeted miR-34a-5p to regulate its expression

### 2.5 lncRNA XIST 通过靶向 miR-34a-5p 上调 SIRT6 表达

通过 Starbase 软件检测发现, SIRT6 与 miR-34a-5p 可直接靶向结合(图 5A)。通过 qPCR 检测发现, 与癌旁组织相比, SIRT6 在 OSCC 患者癌组织中表达明显上调( $t=-18.64, P<0.01$ , 图 5B); 与人口腔正常角质化细胞 hNOK 相比, 在 OSCC 细胞系中表达明显上调(均  $P<0.05$ , 图 5C)。采用双荧光素酶报告基因检测 pmirGLO-SIRT6 WT/MUT 与 miR-34a-5p 模拟物后转染细胞后的荧光素酶活性, 结果显示, 转染 pmirGLO-SIRT6 WT 及 miR-34a-5p 模拟物后显著抑制了

Cal-27 及 Tca-8113 细胞的荧光素酶活性( $t=84.85, 87.68$ , 均  $P<0.01$ , 图 5D、E), 而转染 pmirGLO-SIRT6 MUT 及 miR-34a-5p 模拟物后对细胞的荧光素酶活性无影响( $t=2.83, 4.24, P>0.05$ , 图 5D、E)。qPCR 检测结果显示, 敲降 lncRNA XIST 后明显抑制了 SIRT6 表达(均  $P<0.01$ , 图 5F、G), 而同时转染 miR-34a-5p 抑制剂则逆转了 SIRT6 表达的抑制(均  $P<0.01$ , 图 5F、G)。相关性分析结果显示, lncRNA XIST 与 miR-34a-5p 表达呈负相关( $R^2=0.094, P=0.04$ , 图 5H), SIRT6 与 miR-34a-5p 表达呈负相关( $R^2=0.68, P<0.01$ , 图 5I), lncRNA XIST 与 SIRT6 表达呈正相关( $R^2=$

0.68,  $P < 0.01$ , 图 5J)。结果表明, lncRNA XIST 可通过靶向 miR-34a-5p 上调 SIRT6 表达。



\*\* $P < 0.01$  vs Paracancer group, or hNOK cells group, or shRNA group, or NC inhibitor group, or NC group

A: SIRT6 can directly target miR-34a-5p, detected by Starbase software; B, C: SIRT6 is highly expressed in OSCC tissues and cells; D, E: Transfection of pmirGLO-SIRT6 WT and miR-34a-5p mimics significantly inhibited luciferase activity in Cal-27 and Tca-8113 cells; F, G: Knockdown of lncRNA XIST inhibited SIRT6 expression, and transfection of miR-34a-5p inhibitor reversed this inhibition; H, I, J: Correlation analysis between lncRNA XIST, miR-34a-5p and SIRT6 expression

图 5 miR-34a-5p 与 SIRT6 靶向结合并调控其表达

Fig.5 miR-34a-5p directly targeted SIRT6 to regulate its expression

### 2.6 XIST/miR-34a-5p/SIRT6 分子轴调控 Cal-27 细胞生物学行为

进一步通过 Cal-27 细胞实验验证 XIST/miR-34a-5p/SIRT6 分子轴对 OSCC 细胞增殖 (图 6A)、迁移及侵袭 (图 B、C)、凋亡 (图 6D)、的调控作用。结果显示, 敲降 lncRNA XIST 后明显抑制细胞增殖 ( $P < 0.01$ )、迁移及侵袭 (均  $P < 0.01$ ), 促进了细胞凋亡 ( $P < 0.01$ ); 而同时转染 miR-34a-5p 抑制剂促进了细胞增殖 ( $P < 0.05$ )、迁移 ( $P < 0.01$ ) 及侵袭 ( $P < 0.01$ ), 并抑制细胞凋亡 ( $P < 0.01$ ); 或同时转染 pcDNA-SIRT6 载体也能促进细胞增殖 ( $P < 0.05$ )、迁移 ( $P < 0.01$ ) 及侵袭 ( $P < 0.01$ ), 并抑制细胞凋亡 ( $P < 0.01$ )。

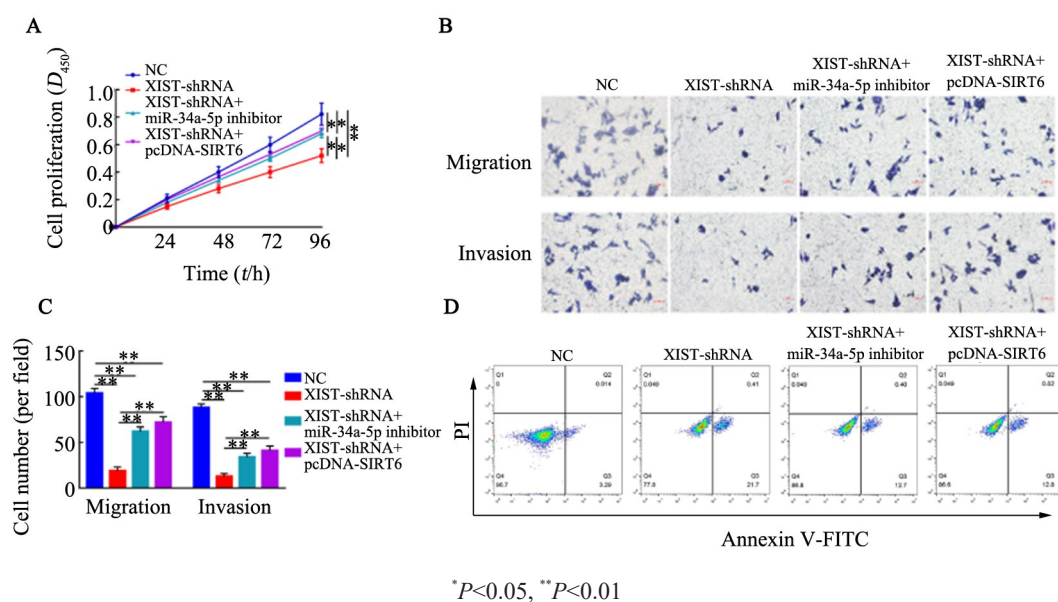
### 3 讨论

lncRNA XIST 在结直肠癌中通过调控细胞转移及上皮间质转化发挥癌基因作用<sup>[20]</sup>, 在非小细胞肺癌中通过抑制 KLF2 基因发挥癌基因作用<sup>[21]</sup>, 通过与 TET1 靶向结合下调 P53 基因表达参与膀胱癌的发展进程<sup>[22]</sup>。在乳腺癌中, lncRNA XIST 通过调控 miR-155/CDX1 分子轴抑制细胞的增殖、迁移及侵袭过程<sup>[23]</sup>。由此可见, lncRNA XIST 在不同的肿瘤中即可扮演癌基因又可扮演抑癌基因。本研究发现, lncRNA XIST 在 OSCC 患者癌组织及细胞系中高表达, 且 lncRNA XIST 的表达能够促进 OSCC 细胞的增殖、迁移及侵袭, 表明 lncRNA XIST 在 OSCC 中

发挥促癌基因作用。

lncRNA 可通过调控 miRNA 及下游的表达调控肿瘤的增殖、迁移及侵袭等,或者通过与 miRNA 内源性竞争吸附靶基因调控其表达进而发挥作用。本研究通过细胞实验证明,在 OSCC 中,lncRNA XIST 通过调控 miR-34a-5p/SIRT6 分子轴调控细胞的增殖、迁移及侵袭。其中 miR-34a-5p 是在肿瘤中研究较多的 miRNA,最重要的机制是可通过调控 P53 基因的表达调控细胞的增殖、衰老、死亡及上皮间质转化过程<sup>[24]</sup>。miR-34a-5p 能在多种肿瘤中发挥抑癌基因作用,如可通过靶向 BCL-2 抑制鼻窦鳞状细胞癌细胞的迁移及侵袭<sup>[25]</sup>,通过抑制双调控蛋白和肿瘤转移抑

制头颈部鳞癌的转移<sup>[26]</sup>,通过靶向 MMP9 和 MMP14 抑制舌鳞癌的迁移和侵袭<sup>[27]</sup>,miR-34a-5p 可通过调控 P53 表达在 OSCC 发挥抑癌基因作用<sup>[28]</sup>。Sirtuin 蛋白家族是依赖 NAD<sup>+</sup> 的蛋白去乙酰化酶和/或单核转移酶<sup>[29]</sup>。这些蛋白质在定位和功能分化上各有不同,SIRT1、2、6 和 7 作为重要的表观遗传修饰的调节器,而 SIRT3、4 和 5 主要在线粒体发挥功能,因此,sirtuins 在发育、细胞分化和衰老过程中的具有关键作用<sup>[30]</sup>。本研究实验证明,lncRNA XIST 可通过抑制 miR-34a-5p,从而促进 SIRT6 表达从而促进 OSCC 细胞的增殖、迁移及侵袭。



A: CCK-8 detected cell proliferation; B, C: Migration and invasion of cells detected by Transwell assay (×100); D: Apoptosis of Cal-27 cells detected by Flow cytometry

图6 lncRNA XIST/miR-34a-5p/SIRT6 分子轴调控 OSCC 细胞生物学行为

Fig.6 lncRNA XIST/miR-34a-5p/SIRT6 axis regulated the biological behavior of OSCC cells

综上所述,本研究发现 lncRNA XIST 在 OSCC 患者癌组织及细胞系中高表达,并通过细胞实验验证 lncRNA XIST 通过调控 miR-34a-5p/SIRT6 分子轴促进 OSCC 细胞的增殖及转移,因此,lncRNA XIST 及 SIRT6 有可能成为 OSCC 诊断及治疗的靶点。

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