# lncRNA SOX2-OT通过调控miR-519e-5p影响乳腺癌细胞增殖及放射敏感性的分子机制研究

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**摘要** 该研究探讨了lncRNA SOX2-OT对乳腺癌细胞增殖和放射敏感性的影响及可能机制。采用qRT-PCR法检测了正常乳腺上皮细胞MCF-10A及乳腺癌细胞系(MCF-7、T47D和Bcap-37)中lncRNA SOX2-OT和miR-519e-5p表达。乳腺癌T47D细胞中lncRNA SOX2-OT和miR-519e-5p表达较MCF-10A细胞差异最显著($P$<0.05), 选择乳腺癌T47D细胞为研究对象。采用核质分离及RNA荧光原位杂交分析lncRNA SOX2-OT在T47D细胞内的定位。分别向T47D细胞转染lncRNA SOX2-OT小干扰RNA、miR-519e-5p模拟物、过表达RNA SOX2-OT、共转染lncRNA SOX2-OT小干扰RNA与miR-519e-5p抑制剂、共转染过表达RNA SOX2-OT与miR-519e-5p模拟物, 即得si-lncRNA SOX2-OT组、si-NC组、miR-519e-5p组、miR-NC组、si-lncRNA SOX2-OT+anti-miR-519e-5p组、si-lncRNA SOX2-OT+anti-miR-NC组、RNA SOX2-OT+miR-519e-5p mimics组、si-lncRNA SOX2-OT+miR-NC组。采用CCK-8法观察细胞增殖活性, 克隆形成实验计算细胞存活分数, 并用单击多靶数学模型计算细胞增敏比, 蛋白质印迹法检测细胞中CyclinD1和γ-H2AX蛋白表达。双荧光素酶报告基因实验验证lncRNA SOX2-OT和miR-519e-5p的调控关系。结果显示, lncRNA SOX2-OT主要定位于T47D的细胞质。敲减lncRNA SOX2-OT或上调miR-519e-5p后, T47D细胞增殖活性和存活分数均降低($P$<0.05), 增敏比分别为1.195、1.343, 细胞中CyclinD1蛋白表达降低($P$<0.05), γ-H2AX蛋白表达升高($P$<0.05)。lncRNA SOX2-OT在T47D细胞中靶向负调控miR-519e-5p。下调或上调miR-519e-5p可分别逆转敲减或上调lncRNA SOX2-OT对T47D细胞增殖和放射敏感性的影响。总之, lncRNA SOX2-OT可能通过靶向负调控miR-519e-5p促进乳腺癌细胞增殖并降低乳腺细胞对放射的敏感性。

**关键词** 乳腺癌; lncRNA SOX2-OT; miR-519e-5p; 细胞增殖; 放射敏感性

# Study On the Molecular Mechanism of LncRNA SOX2-OT Affecting Breast Cancer Cell Proliferation and Radiosensitivity by Regulating miR-519e-5p

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**Abstract** This article explored the effect and possible mechanism of lncRNA SOX2-OT on the proliferation and radiosensitivity of breast cancer cells. The expression of lncRNA SOX2-OT and miR-519e-5p in normal breast epithelial cells MCF-10A and breast cancer cell lines (MCF-7, T47D and Bcap-37) were detected by qRT-PCR. The localization of lncRNA SOX2-OT in T47D cells was analyzed by nucleocytoplasmic isolation and RNA fluorescence *in situ* hybridization. The expression of lncRNA SOX2-OT and miR-519e-5p in breast cancer T47D cells was significantly different from that in MCF-10A cells ($P$<0.05). Breast cancer T47D cells were selected as the research object.The T47D cells were transfected with lncRNA SOX2-OT siRNA, miR-519e-5p mimic, overexpressed RNA SOX2-OT, co-transfected with lncRNA SOX2-OT siRNA and miR-519e-5p inhibitor, co-transfected overexpression RNA SOX2-OT and miR-519e-5p mimics were transfected to obtain si-lncRNA SOX2-OT group, si-NC group, miR-519e-5p group, miR-NC group, si-lncRNA SOX2-OT+anti-miR-519e-5p group, si-lncRNA SOX2-OT+anti-miR-NC group, RNA SOX2-OT+miR-519e-5p mimics group, si-lncRNA SOX2-OT+miR-NC group. Cell proliferation activity was observed by CCK-8 method, cell survival fraction was calculated by colony formation assay, and cell sensitization ratio was calculated by single-click multi-target mathematical model. CyclinD1 and γ-H2AX protein expressions in cells were detected by western blotting. The dual-luciferase reporter gene experiment verified the regulatory relationship between lncRNA SOX2-OT and miR-519e-5p. The results showed that the lncRNA SOX2-OT was mainly localized in the cytoplasm of T47D. The knockdown of lncRNA SOX2-OT or up-regulation of miR-519e-5p, the proliferation activity and survival fraction of T47D cells were both decreased ($P$<0.05), and the sensitization ratios were 1.195 and 1.343, respectively. CyclinD1 protein expression in cells decreased ($P$<0.05), and the expression of γ-H2AX protein increased ($P$<0.05). The lncRNA SOX2-OT targeted and negatively regulated miR-519e-5p in T47D cells. Down-regulation or up-regulation of miR-519e-5p reversed the effects of knock-down or up-regulation of lncRNA SOX2-OT on T47D cell proliferation and radiosensitivity, respectively. In conclusion, the lncRNA SOX2-OT may promote breast cancer cell proliferation and reduce cell radiosensitivity through targeting and negatively regulating miR-519e-5p.

**Keywords** breast cancer; lncRNA SOX2-OT; miR-519e-5p; cell proliferation; radiosensitivity

乳腺癌(breast cancer, BC)是威胁女性生命健康的常见恶性肿瘤, 其治疗方法主要有外科手术、放疗、化疗等, 其中标准模式是外科保乳手术和乳房切除术术后结合放疗, 放射一直在乳腺癌治疗中发挥重要作用, 放射治疗可减少癌细胞远处转移并提高患者长期生存率[1], 但仍有部分乳腺癌患者化疗失败, 主要原因在于乳腺癌细胞的过度增殖及其对放射线的敏感性较低[2], 探究影响乳腺癌细胞增殖及放射敏感性的分子机制可为改善该肿瘤放疗的疗效提供新靶点。SRY-box转录因子2重叠转录物(SRY-box transcription factor 2 overlapping transcript, SOX2-OT)是一种进化上保守的长链非编码RNA(long non-coding RNA, lncRNA), 位于染色体3q26.3, 转录本长度为561 bp, 其内含子区域包含*SOX2*基因, 这是胚胎干细胞多能性的主要调节因子。人类*SOX2-OT*基因包含多个外显子并具有多个转录起始位点可产生数百个转录物[3]。研究显示, lncRNA SOX2-OT可分别通过靶向miR-192-5p/RAB2A、miR-452-5p/HMGB3轴促进胶质母细胞瘤和前列腺癌细胞生长[4-5]。然而, lncRNA SOX2-OT对乳腺癌发生发展的影响和机制还未知。Starbase靶基因在线软件预测显示, lncRNA SOX2-OT与miR-519e-5p存在靶向调控关系。miR-519e-5p在甲状腺癌和宫颈癌中均表达下调, 上调miR-519e-5p可分别靶向抑制YWHAH、Notch2的表达, 进而降低甲状腺癌和宫颈癌细胞的恶性增殖, 抑制恶性肿瘤的发展[6-7]。但miR-519e-5p对乳腺癌发生发展的影响也还未知。本研究拟探究lncRNA SOX2-OT和miR-519e-5p对乳腺癌细胞增殖和放射敏感性的影响, 以期为改善乳腺癌放疗疗效提供分子靶点。

## 1 资料与方法

### 1.1 细胞和试剂

正常乳腺上皮细胞MCF-10A及乳腺癌细胞系

(MCF-7、T47D和Bcap-37)购自中国科学院上海细胞库; PCR实验相关试剂盒购自大连宝生物工程有限公司; 胎牛血清购自浙江天杭生物科技股份有限公司; CCK-8试剂盒、RPMI 1640培养液和双荧光素酶活性检测试剂盒购自北京索莱宝科技有限公司; Lipofectamine™ 2000试剂盒购自美国Invitrogen公司; CyclinD1、γ-H2AX和β-actin抗体购自Abcam公司; 引物序列、野生型(WT)和突变型(MUT) lncRNA SOX2-OT荧光素酶报告基因载体、lncRNA SOX2-OT小干扰RNA(si-lncRNA SOX2-OT)及阴性对照序列(si-NC)、miR-519e-5p模拟物(mimcs)和抑制剂(anti-miR-519e-5p)、模拟对照序列(miR-NC)和抑制剂阴性对照序列(anti-miR-NC)、lncRNA SOX2-OT过表达(RNA SOX2-OT)和阴性对照(RNA SOX2-OT-NEG)购自上海生工生物工程有限公司; 核质分提试剂盒(Ambion PARISTM Kit, Cat No.AM1921)购自美国Thermo Fisher公司; 多通道二代荧光试剂盒购自美国Advanced Cell Diagnostics公司; 荧光标记的lncRNA探针购自美国Advanced Cell Diagnostics公司。

## 1.2 方法

1.2.1 *细胞培养* 复苏MCF-10A、MCF-7、T47D和Bcap-37细胞, 并将其放入5% $CO_2$培养箱中用含有10%胎牛血清的RPMI 1640培养液(完全培养液)进行培养。

1.2.2 *qRT-PCR法检测lncRNA SOX2-OT和miR-519e-5p表达* 将对数期MCF-10A、MCF-7、T47D和Bcap-37细胞均接种至6孔板(细胞浓度5.0×10$^4$个/mL, 2.5 mL/孔)中。培养24 h后, 收集细胞。采用RNA提取试剂盒获得细胞中总RNA, 将其利用逆转录试剂盒逆转录为cDNA后, 行PCR反应。引物序列见表1。$2^{-\Delta\Delta Ct}$法计算lncRNA SOX2-OT相对*β-actin*、miR-519e-5p相对*U6*的表达量。选择较MCF-10A细胞lncRNA SOX2-OT和miR-519e-5p表达差异最显著的乳腺癌T47D细胞进行后续实验。

1.2.3 *核质分离及RNA荧光原位杂交*[8] 使用核质分提试剂盒分离T47D细胞核质, 采用荧光试剂盒进行原位杂交, 荧光显微镜下观察拍照, 考察lncRNA SOX2-OT是否定位于细胞质。

1.2.4 *细胞转染* 于96孔板中分别接种T47D细胞(细胞浓度5.0×10$^4$个/mL, 2.5 mL/孔), 将Lipofectamine™ 2000试剂分别与si-lncRNA SOX2-OT(si-lncRNA SOX2-OT组)、si-NC(si-NC组)、miR-519e-5p mimics(miR-519e-5p组)、miR-NC(miR-NC组)、lncRNA SOX2-OT过表达(RNA SOX2-OT)、阴性对照(RNA SOX2-OT-NEG)、si-lncRNA SOX2-OT和anti-miR-519e-5p(si-lncRNA SOX2-OT+anti-miR-519e-5p组)、si-lncRNA SOX2-OT和anti-miR-NC(si-lncRNA SOX2-OT+anti-miR-NC组)、RNA SOX2-OT和miR-519e-5p mimics (RNA SOX2-OT+miR-519e-5p mimics组)、RNA SOX2-OT和miR-NC(si-lncRNA SOX2-OT+miR-NC组)混合均匀, 37 °C孵育细胞12 h后, 换为完全培养液, 再培养24 h, 备用。采用1.2.2项下方法检测细胞中lncRNA SOX2-OT或miR-519e-5p表达验证转染效果。

1.2.5 *CCK-8法检测细胞增殖活性* 于96孔板中接种各组细胞(细胞浓度5.0×10$^4$个/mL, 2.5 mL/孔), 培养24 h后, 每孔加10 μL CCK-8, 37 °C孵育细胞2 h后, 用酶标仪检测各孔吸光度(*D*)值。每组设3个复孔并进行3次独立重复实验。

1.2.6 *克隆形成实验*[9] 于96孔板中接种各组细胞(细胞浓度5.0×10$^4$个/mL, 2.5 mL/孔)。置于6MV-X线直线加速器下进行照射。照射条件: 源皮距100 cm, 剂量率200 cGy/min, 视野为15 cm×15 cm, 吸收剂量分别为0、2、4、6、8 Gy。照射后, 置于培养箱中培养14天。弃培养液, 先置于多聚甲醛溶液中进行固定(37 °C), 时间为25 min。然后再使用结晶紫在同样温度条件下进行染色, 时间为15 min。用PBS清洗后, 显微镜观察, 统计超过50个细胞的克隆。克隆

**表1 引物序列**

**Table 1 Primer sequences**

| 基因名称<br>Gene name | 上游<br>Forward | 下游<br>Reverse |
|---|---|---|
| lncRNA SOX2-OT | 5′-GTT CAT GGC CTG GAC TCT CC-3′ | 5′-ATT GCT AGC CCT CAC ACC TC-3′ |
| *β-actin* | 5′-CTG GAA CGG TGA AGG TGA C-3′ | 5′-CGG CCA CAT TGT GAA CTT TG-3′ |
| miR-519e-5p | 5′-CGA TAG GCG CTG AGC GGA C-3′ | 5′-CGT AGT CGT AGC TAG CTG C-3′ |
| *U6* | 5′-ACC CTG AGA AAT ACC CTC ACA T-3′ | 5′-GAC GAC TGA GCC CCT GAT G-3′ |

形成率(PE)=克隆数/接种细胞数×100%。存活分数(SF)=$PE_{实验组}/PE_{对照组}$×100%。依据单击多靶模型, 使用GraphPad Prism 5.0软件绘制细胞存活曲线。依据$SF=1-(1-e^{-D/D_0})^N$对数据进行拟合, 其中D为照射剂量, N为外推数, $D_0$为平均致死剂量, Dq为准阈剂量。计算放射增敏比(SER), $SER=D_{0对照组}/D_{0实验组}$。每组设3个复孔并进行3次独立重复实验。

1.2.7 蛋白质印迹法检测细胞中CyclinD1和γ-H2AX蛋白表达 ① 于6孔板中接种各组细胞(细胞浓度5.0×10⁴个/mL, 2.5 mL/孔)。培养24 h后, 收集各组细胞, 检测细胞中CyclinD1蛋白表达; ② 于6孔板中接种各组细胞(细胞浓度5.0×10⁴个/mL, 2.5 mL/孔)。使用4 Gy照射细胞后, 检测细胞中γ-H2AX蛋白表达。细胞中总蛋白的获取采用RIPA试剂, 然后利用BCA试剂盒检测蛋白浓度。行SDS-PAGE电泳实验对总蛋白进行分离, 并转至PVDF膜, 37 ℃条件下用5%脱脂奶粉封闭2 h。于4 ℃冰箱中分别用稀释后的CyclinD1(1:1 000)、γ-H2AX(1:500)、β-actin(1:1 000)一抗孵育过夜, 洗膜后, 再于室温下用稀释的山羊抗兔二抗(1:2 000)孵育1 h。加显影液, 避光显影, 曝光拍照, ImageJ软件分析CyclinD1、γ-H2AX相对β-actin的表达量。每组设3个复孔并进行3次独立重复实验。

1.2.8 双荧光素酶报告基因实验 于6孔板中接种T47D细胞(细胞浓度5.0×10⁴个/mL, 2.5 mL/孔)。将Lipofectamine™ 2000试剂分别与WT-lncRNA SOX2-OT和miR-519e-5p mimics(或miR-NC)、MUT-lncRNA SOX2-OT和miR-519e-5p mimics(或miR-NC)混合均匀, 每孔100 μL加入6孔板中, 37 ℃孵育细胞12 h后, 弃培养液, 裂解细胞。用半径10 cm离心机将裂解液离心(3 500 r/min, 10 min)后, 取20 μL上清液, 加100 μL 1× 萤火虫或海肾荧光素酶反应工作液, 混匀后, 检测萤火虫或海肾的荧光强度, 以萤火虫与海肾荧光强度的比值表示细胞荧光素酶活性。

### 1.3 统计学分析

SPSS 22.0软件进行统计学分析。以均数±标准差($\bar{x}\pm s$)表示计量资料。两组间比较用独立样本$t$检验; 多组间比较用单因素方差分析, 进一步两组间比较用LSD-$t$检验。以$P<0.05$表示差异有统计学意义。

## 2 结果

### 2.1 乳腺癌细胞系中lncRNA SOX2-OT和miR-519e-5p的表达

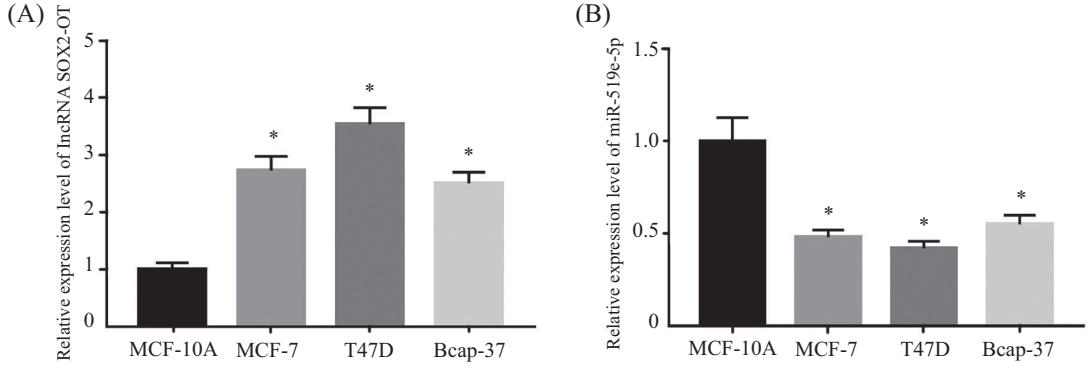
与MCF-10A细胞比较, 乳腺癌细胞系(MCF-7、T47D和Bcap-37)中lncRNA SOX2-OT表达水平升高($P<0.05$, 图1A), 而miR-519e-5p表达水平降低($P<0.05$, 图1B), 且乳腺癌T47D细胞中lncRNA SOX2-OT和miR-519e-5p表达较MCF-10A细胞差异最显著($P<0.05$)。

### 2.2 乳腺癌细胞系中lncRNA SOX2-OT和miR-519e-5p的表达

核质分离和RNA原位杂交实验显示, lncRNA SOX2-OT主要定位于T47D的细胞质(图2)。

### 2.3 敲减lncRNA SOX2-OT对乳腺癌T47D细胞增殖和放射敏感性的影响

NC组、si-NC组和si-lncRNA SOX2-OT组T47D细胞中lncRNA SOX2-OT的表达量分别为1.00±0.11、




A: 乳腺癌细胞系中lncRNA SOX2-OT的表达; B: 乳腺癌细胞系中miR-519e-5p的表达。*$P<0.05$, 与MCF-10A比较。

A: the expression of lncRNA SOX2-OT in breast cancer cell lines; B: the expression of miR-519e-5p in breast cancer cell lines. $P<0.05$ compared with MCF-10A.

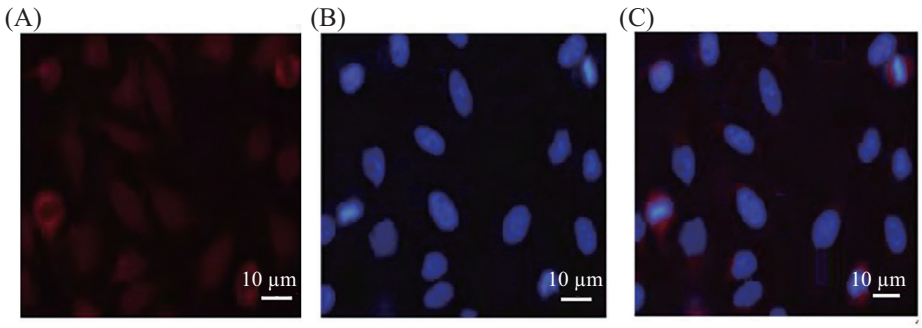
**图1 乳腺癌细胞系中lncRNA SOX2-OT和miR-519e-5p的表达**

**Fig.1 The expression of lncRNA SOX2-OT and miR-519e-5p in breast cancer cell lines**

1.02±0.10、0.46±0.04, 三组间lncRNA SOX2-OT表达量比较差异具有统计学意义($F$=114.987, $P$<0.05), si-lncRNA SOX2-OT组T47D细胞中lncRNA SOX2-OT的表达量较NC组和si-NC组被敲减。si-lncRNA SOX2-OT组T47D细胞增殖活性、分活分数及细胞中CyclinD1蛋白表达均低于NC组和si-NC组($P$<0.05), γ-H2AX蛋白表达高于NC组和si-NC组($P$<0.05(图3和表2)。si-lncRNA SOX2-OT组T47D细胞增敏比为1.195(表3)。

### 2.4 上调miR-519e-5p对乳腺癌T47D细胞增殖和放射敏感性的影响

miR-NC组、miR-519e-5p组T47D细胞中miR-519e-5p的表达量分别为1.00±0.09、2.61±0.24, 两组间miR-519e-5p表达量比较差异具有统计学意义($t$=18.844, $P$<0.05), miR-519e-5p组T47D细胞中miR-519e-5p的表达较miR-NC组上调。miR-519e-5p组T47D细胞增殖活性、存活分数及细胞中CyclinD1

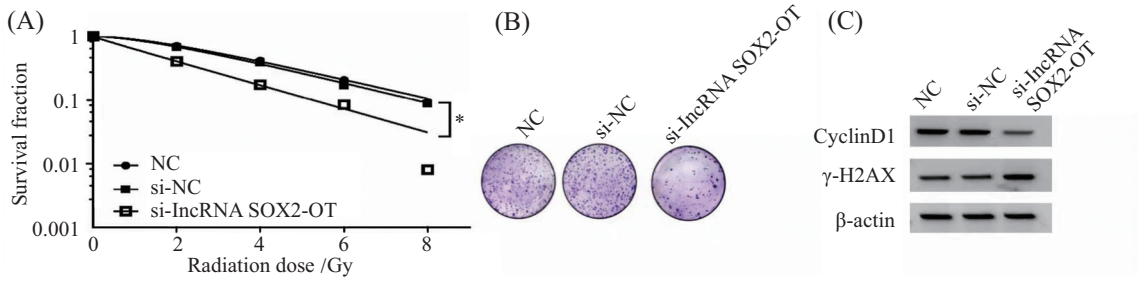



A: lncRNA SOX2-OT RNA表达(红色荧光); B: 细胞质(蓝色荧光); C: 合并的荧光

A: RNA expression of lncRNA SOX2-OT (red fluorescence); B: cell cytoplasm (blue fluorescence); C: the merged fluorescence.

**图2 lncRNA SOX2-OT定位于T47D细胞质**

**Fig.2 The cytoplasm locatization of lncRNA SOX2-OT in T47D**




A: 细胞存活分数; B: 克隆形成实验; C: Western Blot检测CyclinD1和γ-H2AX蛋白表达。*$P$<0.05, 与si-NC组和NC组比较。

A: cell survival fraction; B: clone formation assay; C: Western blot detected the expression of CyclinD1 and γ-H2AX protein. *$P$<0.05 compared with si-NC and NC.

**图3 敲减lncRNA SOX2-OT对T47D细胞放射敏感性的影响**

**Fig.3 Effect of knocking down lncRNA SOX2-OT on radiosensitivity of T47D cells**

**表2 敲减lncRNA SOX2-OT对T47D细胞增殖及放射敏感性的影响**

**Table 2 Effects of knocking down lncRNA SOX2-OT on proliferation and radiosensitivity of T47D cells**

| 组别 Group | CyclinD1 | γ-H2AX | D |
|---|---|---|---|
| NC | 0.86±0.07 | 0.37±0.03 | 1.124±0.10 |
| si-NC | 0.84±0.08 | 0.36±0.03 | 1.121±0.09 |
| si-lncRNA SOX2-OT | 0.41±0.04* | 0.75±0.07* | 0.561±0.05* |
| $F$ | 135.279 | 199.209 | 137.748 |
| $P$ | 0.000 | 0.000 | 0.000 |

*$P$<0.05, 与si-NC组和NC组比较; $\bar{x}\pm s$, $n$=9。

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

蛋白表达均低于miR-NC组($P$<0.05), γ-H2AX蛋白表达高于miR-NC组($P$<0.05)(图4和表4)。miR-519e-5p组T47D细胞增敏比为1.343(表5)。

### 2.5 lncRNA SOX2-OT靶向调控miR-519e-5p

Starbase靶基因在线软件预测显示的lncRNA SOX2-OT与miR-519e-5p的结合位点见图5A。共转染WT-lncRNA SOX2-OT与miR-519e-5p mimics的T47D细胞荧光素酶较共转染WT-lncRNA SOX2-OT与miR-519e-5p mimics的T47D细胞显著降低($t$=14.353, $P$<0.05), 共转染MUT-lncRNA SOX2-OT与miR-519e-5p mimics的T47D细胞荧光素酶活性与共转染MUT-lncRNA SOX2-OT与miR-519e-5p mimics的T47D细胞比较差异无统计学意义($t$=0.446, $P$=0.662)(图5B), 说明lncRNA SOX2-OT可靶向结合miR-519e-5p。si-NC组和si-lncRNA SOX2-OT组T47D细胞中miR-519e-5p的表达量分别为1.00±0.12、2.04±0.17, 两组间比较差异具有统计学意义($t$=14.944, $P$<0.05), 说明敲减lncRNA SOX2-OT可促进T47D细胞中miR-519e-5p的表达。

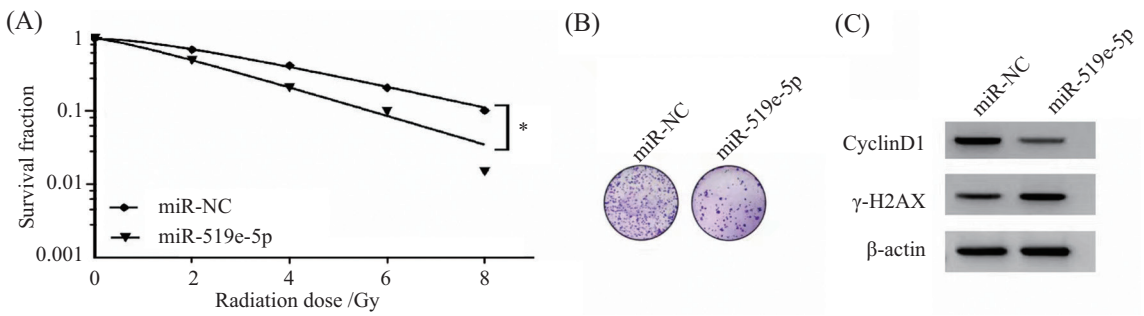
### 2.6 下调miR-519e-5p逆转敲减lncRNA SOX2-OT对T47D细胞增殖和放射敏感性的影响

si-lncRNA SOX2-OT+anti-miR-NC组、si-lncRNA SOX2-OT+anti-miR-519e-5p组T47D细胞中miR-519e-5p的表达量分别为1.00±0.08、0.43±0.04, 两组间miR-519e-5p表达量比较差异具有统计学意义($t$=19.118, $P$<0.05), si-lncRNA SOX2-OT+anti-

表3 敲减lncRNA SOX2-OT后单击多靶数学模型参数

Table 3 Parameters of single-click multi-target mathematical model after knockdown of lncRNA SOX2-OT

| 组别 Group | $D_0$ /Gy | $D_q$ /Gy | N | SF2 | k | 放射增敏比 SER |
|---|---|---|---|---|---|---|
| NC | 2.805 | 1.774 | 1.882 | 0.719 | 0.357 | 1.000 |
| si-NC | 2.690 | 1.591 | 1.807 | 0.688 | 0.372 | 1.043 |
| si-lncRNA SOX2-OT | 2.348 | 0.142 | 0.941 | 0.408 | 0.426 | 1.195 |




A: 细胞存活分数; B: 克隆形成实验; C: Western blot检测CyclinD1、γ-H2AX蛋白表达; *$P$<0.05, 与miR-NC组比较。

A: cell survival fraction; B: clone formation assay; C: Western blot detected the expression of CyclinD1 and γ-H2AX protein; *$P$<0.05 compared with miR-NC.

图4 上调miR-519e-5p对T47D细胞放射敏感性的影响

Fig.4 Effect of up-regulating miR-519e-5p on radiosensitivity of T47D cells

表4 上调miR-519e-5p对T47D细胞增殖及放射敏感性的影响

Table 4 Effects of up-regulating miR-519e-5p on proliferation and radiosensitivity of T47D cells

| 组别 Group | CyclinD1 | γ-H2AX | D |
|---|---|---|---|
| miR-NC | 0.85±0.08 | 0.35±0.03 | 1.124±0.10 |
| miR-519e-5p | 0.43±0.04* | 0.71±0.07* | 0.614±0.05* |
| $t$ | 14.087 | 14.181 | 13.685 |
| $P$ | 0.000 | 0.000 | 0.000 |

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

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

miR-519e-5p组T47D细胞中miR-519e-5p的表达较si-lncRNA SOX2-OT+anti-miR-NC组下调。si-lncRNA SOX2-OT+anti-miR-519e-5p组T47D细胞增殖活性、存活分数及细胞中CyclinD1蛋白表达均高于si-lncRNA SOX2-OT+anti-miR-NC组($P$<0.05), γ-H2AX蛋白表达低于si-lncRNA SOX2-OT+anti-miR-NC组($P$<0.05)(图6和表6)。si-lncRNA SOX2-OT+anti-miR-519e-5p组T47D细胞增敏比为0.775(表7)。

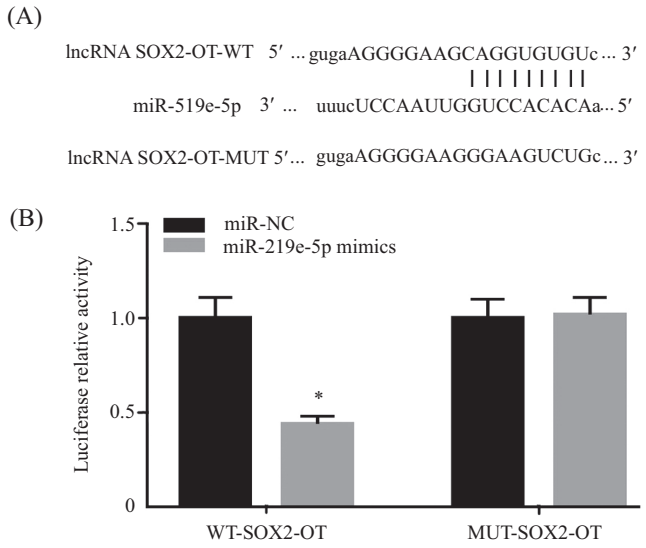
### 2.7 上调miR-519e-5p逆转上调lncRNA SOX2-OT对T47D细胞增殖和放射敏感性的影响

RNA SOX2-OT+miR-NC组、RNA SOX2-

表5 上调miR-519e-5p后单击多靶数学模型参数

Table 5 Parameters of single-click multi-target mathematical model after up-regulation of miR-519e-5p

| 组别 Group | $D_0$ /Gy | $D_q$ /Gy | N | SF2 | k | 放射增敏比 SER |
|---|---|---|---|---|---|---|
| miR-NC | 2.941 | 1.663 | 1.760 | 0.712 | 0.340 | 1.000 |
| miR-519e-5p | 2.190 | 0.680 | 1.364 | 0.503 | 0.457 | 1.343 |

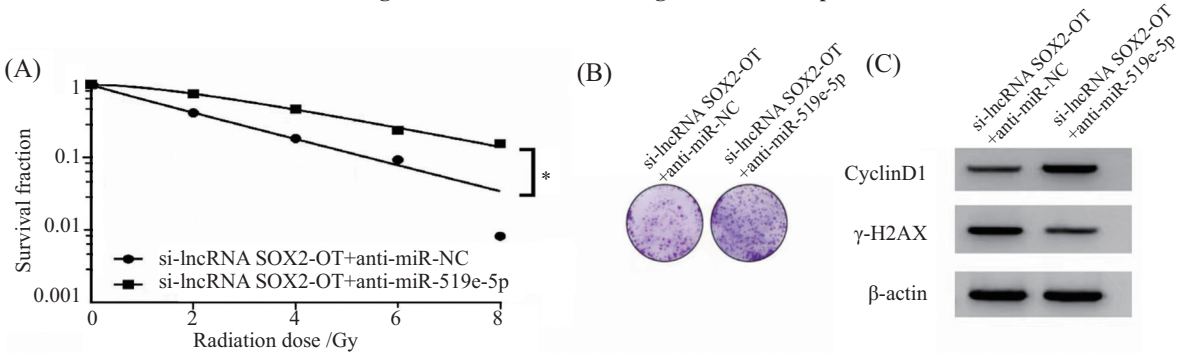



A: lncRNA SOX2-OT与miR-519e-5p的结合位点; B: 双荧光素酶报告实验。*$P$<0.05, 与miR-NC比较。

A: the binding sites of lncRNA SOX2-OT to miR-519e-5p; B: dual luciferase reporter assay. *$P$<0.05 compared with miR-NC.

图5 lncRNA SOX2-OT靶向miR-519e-5p

Fig.5 lncRNA SOX2-OT targets miR-519e-5p




A: 细胞存活分数; B: 克隆形成实验; C: Western blot检测细胞中CyclinD1和γ-H2AX蛋白表达。*$P$<0.05, 与si-lncRNA SOX2-OT+anti-miR-NC比较。

A: cell survival fraction; B: clone formation assay; C: Western blot detected the expression of CyclinD1 and γ-H2AX protein in cells. *$P$<0.05 compared with si-lncRNA SOX2-OT+anti-miR-NC.

图6 下调miR-519e-5p逆转敲减lncRNA SOX2-OT对T47D细胞放射敏感性的影响

Fig.6 Down-regulation of miR-519e-5p reversed the effect of knocking down lncRNA SOX2-OT on the radiosensitivity of T47D cells

**表6 下调miR-519e-5p 逆转敲减lncRNA SOX2-OT对T47D细胞增殖和放射敏感性的影响**

**Table 6 Down-regulation of miR-519e-5p reversed the effect of knocking down lncRNA SOX2-OT on the proliferation and radiosensitivity of T47D cells**

| 组别 Group | CyclinD1 | γ-H2AX | D |
|---|---|---|---|
| si-lncRNA SOX2-OT+anti-miR-NC | 0.42±0.04 | 0.74±0.07 | 0.567±0.05 |
| si-lncRNA SOX2-OT+anti-miR-519e-5p | 0.83±0.08* | 0.34±0.03* | 1.088±0.10* |
| *t* | 13.752 | 15.757 | 13.980 |
| *P* | 0.000 | 0.000 | 0.000 |

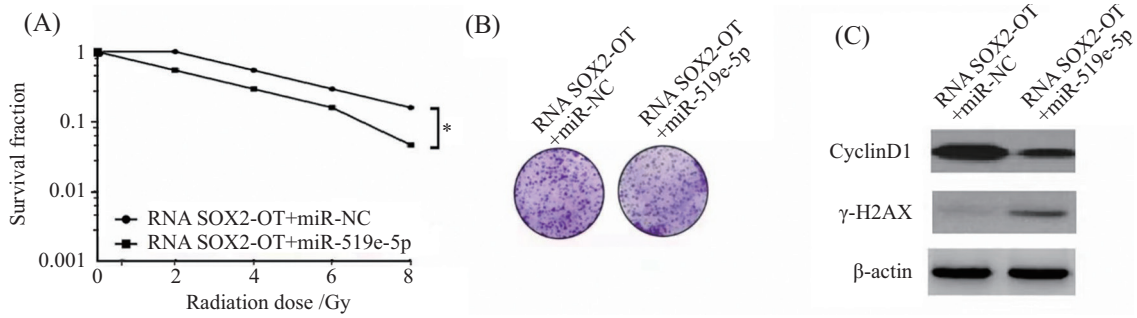
*$P$<0.05, 与si-lncRNA SOX2-OT+anti-miR-NC比较; $\bar{x}\pm s$, $n$=9。

*$P$<0.05 compared with si-lncRNA SOX2-OT+anti-miR-NC; $\bar{x}\pm s$, $n$=9.

**表7 下调miR-519e-5p后单击多靶数学模型参数**

**Table 7 Parameters of single-click multi-target mathematical model after down-regulation of miR-519e-5p**

| 组别 Group | $D_0$ /Gy | $D_q$ /Gy | N | SF2 | k | 放射增敏比 SER |
|---|---|---|---|---|---|---|
| si-lncRNA SOX2-OT+anti-miR-NC | 2.430 | 0.207 | 0.918 | 0.412 | 0.412 | 1.000 |
| si-lncRNA SOX2-OT+anti-miR-519e-5p | 3.137 | 1.916 | 1.842 | 0.750 | 0.319 | 0.775 |




A: 细胞存活分数; B: 克隆形成实验; C: Western Blot检测细胞中CyclinD1和γ-H2AX蛋白表达。*$P$<0.05, 与si-lncRNA SOX2-OT+anti-miR-NC比较。

A: cell survival fraction; B: clone formation assay; C: Western blot detected the expression of CyclinD1 and γ-H2AX protein in cells. *$P$<0.05 compared with si-lncRNA SOX2-OT+anti-miR-NC.

**图7 上调miR-519e-5p逆转上调lncRNA SOX2-OT对T47D细胞放射敏感性的影响**

**Fig.7 Up-regulation of miR-519e-5p reversed the effect of up-regulating lncRNA SOX2-OT on the radiosensitivity of T47D cells**

OT+miR-519e-5p mimics组T47D细胞中miR-519e-5p的表达量分别为1.00±0.07、2.65±0.22, 两组间miR-519e-5p表达量比较差异具有统计学意义($t$=21.441, $P$<0.05), RNA SOX2-OT+miR-519e-5p mimics组T47D细胞中miR-519e-5p的表达较RNA SOX2-OT+miR-NC组上调。RNA SOX2-OT+miR-519e-5p mimics组T47D细胞增殖活性、存活分数及细胞中CyclinD1蛋白表达均低于RNA SOX2-OT+miR-NC组($P$<0.05), γ-H2AX蛋白表达高于RNA SOX2-OT+miR-NC组($P$<0.05)(图7和表8)。si-lncRNA SOX2-OT+anti-miR-519e-5p组T47D细胞增敏比为1.019(表9)。

## 3 讨论

lncRNA是一类长链非编码RNA, 其可发挥miRNA分子海绵作用调控miRNA靶基因的表达, 进而影响肿瘤细胞恶性行为及放射敏感性[10-11]。既往研究显示, 多种lncRNA参与调控乳腺癌细胞的恶性行为及放射敏感性。ZHANG等[12]研究显示, lncRNA HOTAIR在乳腺癌中起致癌基因作用, 其可通过靶向miR-449b-5p促进HSPA1A的表达, 进而降低乳腺癌细胞对放射线的敏感性, 增强放疗抵抗。LIU等[13]研究显示, LINC00511在乳腺癌组织中表达增加, 敲减其表达通过靶向miR-185/STXBP4轴抑制了乳腺癌细胞增殖, 促进了细胞凋亡, 并增强了细胞放

**表8 上调miR-519e-5p 逆转上调lncRNA SOX2-OT对T47D细胞增殖和放射敏感性的影响**

**Table 8 Up -regulation of miR-519e-5p reversed the effect of up-regulating lncRNA SOX2-OT on the proliferation and radiosensitivity of T47D cells**

| 组别 Group | CyclinD1 | γ-H2AX | D |
|---|---|---|---|
| RNA SOX2-OT+miR-NC | 1.56±0.08 | 0.12±0.03 | 1.265±0.103 |
| RNA SOX2-OT+miR-519e-5p mimics | 0.92±0.07* | 0.37±0.04* | 1.135±0.082* |
| *t* | 18.062 | 15.00 | 2.962 |
| *P* | 0.000 | 0.000 | 0.018 |

*$P$<0.05, 与RNA SOX2-OT+miR-NC比较; $\bar{x}\pm s$, $n$=9。

*$P$<0.05 compared with RNA SOX2-OT+miR-NC; $\bar{x}\pm s$, $n$=9.

**表9 上调miR-519e-5p后单击多靶数学模型参数**

**Table 9 Parameters of single-click multi-target mathematical model after up-regulation of miR-519e-5p**

| 组别 Group | $D_0$ /Gy | $D_q$ /Gy | N | SF2 | k | 放射增敏比 SER |
|---|---|---|---|---|---|---|
| RNA SOX2-OT+miR-NC | 3.062 | 1.795 | 1.942 | 0.761 | 0.369 | 1.000 |
| RNA SOX2-OT+miR-519e-5p mimics | 2.812 | 1.421 | 1.601 | 0.624 | 0.321 | 1.019 |

射敏感性。

作为一种lncRNA, lncRNA SOX2-OT在多种肿瘤中发挥调控作用。研究显示, 喉癌细胞中lncRNA SOX2-OT表达量增加, 沉默lncRNA SOX2-OT引起喉癌细胞增殖、侵袭及迁移受阻, 而凋亡加剧, 其作用机制与靶向调控miR-369-3p/CFL2轴有关; 前列腺癌中lncRNA SOX2-OT通过靶向miR-369-3p/CFL2轴促进癌细胞增殖和迁移[14]; lncRNA SOX2-OT在鼻咽癌组织样本及细胞系中均表达水平升高, 且lncRNA SOX2-OT高表达的患者预后不良, 其通过发挥miR-146b-5p分子海绵作用上调HNRNPA2B1的表达, 进而促进了鼻咽癌的发展进程[15]。本实验数据显示, lncRNA SOX2-OT作为分子海绵吸附miRNA的前提在于lncRNA SOX2-OT主要定位于细胞质中, 本研究结果证实了上述论点, 即核质分离及RNA荧光原位杂交分析lncRNA SOX2-OT在T47D细胞内的定位。进一步, lncRNA SOX2-OT在乳腺癌细胞系中的表达明显高于正常乳腺上皮细胞, 与上述相关报道的lncRNA SOX2-OT在喉癌、前列腺癌和鼻咽癌中表达水平升高的结果一致, 这提示lncRNA SOX2-OT也可能作为促癌基因促进乳腺癌的发展进程。

乳腺癌细胞的异常增殖是其发生发展的主要原因, 抑制乳腺癌细胞的增殖对该肿瘤的治疗十分重要。CyclinD1参与调控细胞周期进程, 其可促进细胞周期由$G_1$期进入S期, 加速细胞增殖[16]。本研究结果显示, 敲减lncRNA SOX2-OT降低了乳腺癌T47D细胞的增殖活性及细胞中CyclinD1蛋白表达水平, 这提示敲减lncRNA SOX2-OT可能通过阻碍乳腺癌细胞的细胞周期进程来抑制其增殖。放疗抵抗是乳腺癌放疗失败的主要原因, 增强乳腺癌细胞的放疗敏感性有利于提高放疗疗效[17]。肿瘤细胞经放射线处理后, DNA双链断裂。在双链断裂早期, 位于DNA损伤位点处的组蛋白H2AX被激活并发生磷酸化, 生成γ-H2AX[18]。因此, 检测放射线处理的肿瘤细胞中γ-H2AX的表达可判断DNA损伤情况。本研究结果显示, 敲减lncRNA SOX2-OT降低了放射线照射后乳腺癌T47D细胞的存活分数, 增敏比为1.195, 同时促进了细胞中γ-H2AX蛋白表达, 这说明敲减lncRNA SOX2-OT增强了乳腺癌细胞的放射敏感性, 提示lncRNA SOX2-OT有可能成为改善乳腺癌放疗抵抗的分子靶点。

为了进一步探究敲减lncRNA SOX2-OT抑制乳腺癌细胞增殖并增强其放射敏感性的分子机制, 本研究证实了lncRNA SOX2-OT可靶向结合并负调控miR-519e-5p表达, miR-519e-5p在乳腺癌细胞系中均呈低表达, 上调miR-519e-5p降低了乳腺癌细胞的增殖能力, 同时增强了乳腺癌细胞的放射敏感性, 增敏比为1.343, 这提示miR-519e-5p也可作为改善乳腺癌放疗抵抗的分子靶点; 下调miR-519e-5p逆转了敲减lncRNA SOX2-OT对乳腺癌细胞增殖的抑制作用及放射敏感性的增强作用, 同时, 上调miR-519e-5p逆转了过表达lncRNA SOX2-OT对乳腺癌细胞增殖

的抑制作用及放射敏感性的增强作用, 进一步验证了lncRNA SOX2-OT可能通过靶向负调控miR-519e-5p来影响乳腺癌细胞的增殖及放疗抵抗, 但其调控miR-519e-5p下游靶基因及其功能等的具体机制还有待进一步探究。

综上, 乳腺癌细胞系中lncRNA SOX2-OT表达升高, 而miR-519e-5p表达降低; 敲减lncRNA SOX2-OT对乳腺癌细胞的增殖具有显著抑制作用, 对其放射敏感性起增强作用, 这可能与敲减lncRNA SOX2-OT促进了乳腺癌细胞中miR-519e-5p的表达有关, lncRNA SOX2-OT/miR-519e-5p轴可能为改善乳腺癌放疗抵抗提供了新的分子靶点, 但尚需进一步于动物研究进行验证。

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