LINC00963靶向miR-1224-5p调控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 这项研究探讨长基因间非编码RNA 00963(long intergene noncoding RNA00963, LINC00963)通过靶向miR-1224-5p调控乳腺癌细胞增殖及放射敏感性的分子机制。使用qRT-PCR检测乳腺上皮细胞MCF-10A和乳腺癌细胞(MDA-MB-231、MDA-MB-468、MCF-7)中LINC00963和miR-1224-5p的相对表达情况。将MDA-MB-231细胞分为si-NC组(转染si-NC)、si-LINC00963组(转染si-LINC00963)、miR-NC组(转染miR-NC)、miR-1224-5p组(转染miR-1224-5p)、si-LINC00963+anti-miR-NC组(共转染si-LINC00963和anti-miR-NC)、si-LINC00963+anti-miR-1224-5p组(共转染si-LINC00963和anti-miR-1224-5p)。MTT检测细胞增殖情况; Western blot检测细胞周期素D1(CyclinD1)、增殖细胞核抗原(proliferating cell nuclear antigen, PCNA)蛋白表达情况; 克隆实验检测细胞放射敏感性; 双荧光素酶报告实验检测LINC00963和miR-1224-5p的靶向关系。在乳腺癌细胞中LINC00963相对表达量明显增加, miR-1224-5p相对表达量显著降低; 抑制LINC00963及过表达miR-1224-5p降低乳腺癌细胞增殖活性和细胞存活分数, 并下调CyclinD1、PCNA蛋白表达水平。LINC00963靶向调控miR-1224-5p, 干扰miR-1224-5p可逆转抑制LINC00963表达对乳腺癌细胞增殖和放射敏感性的影响。LINC00963通过靶向抑制miR-1224-5p增加乳腺癌细胞放射敏感性, 并抑制细胞增殖。

关键词 LINC00963; miR-1224-5p; 乳腺癌; 增殖; 放射敏感性

Molecular Mechanism of LINC00963 Targeting miR-1224-5p Regulating Proliferation and Radiosensitivity of Breast Cancer Cells

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Abstract This study investigates the molecular mechanisms of LINC00963 targeting miR-1224-5p regulating proliferation and radiosensitivity of breast cancer cells. qRT-PCR was used to detect the relative expression of LINC00963 and miR-1224-5p in breast epithelial cells MCF-10A and breast cancer cells (MDA-MB-231, MDA-MB-468, MCF-7). Divide MDA-MB-231 cells into si-NC group (transfected with si-NC), si-LINC00963 group (transfected with si-LINC00963), miR-NC group (transfected with miR-NC), miR-1224-5p group (transfected with miR-1224-5p), si-LINC00963+anti-miR-NC group (co-transfected with si-LINC00963 and anti-miR-NC), si-LINC00963+anti-miR-1224-5p group (co-transfected with si-LINC00963 and anti-miR-1224-5p). The expression of LINC00963 and miR-1224-5p was detected by qRT-PCR assay. CyclinD1 and PCNA protein levels were assessed by Western blot assay. Cell proliferation was measured using MTT assay. Cell radiosensitivity was detected

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by cloning assay. The targeting relationship between LINC00963 and miR-1224-5p was confirmed using a dual luciferase reporter experiment. In breast cancer cells, the relative expression of LINC00963 was significantly increased, and the relative expression of miR-1224-5p was significantly reduced. Inhibition of LINC00963 and overexpression of miR-1224-5p reduced breast cancer cell proliferation activity and cell survival score, and down-regulate CyclinD1, PCNA protein expression. miR-1224-5p acted as a target of LINC00963. Interference miR-1224-5p reversed the effects of LINC00963 knockdown on breast cancer cell proliferation and radiosensitivity. LINC00963 might improve breast cancer cell radiosensitivity and suppress cell proliferation by targeting miR-1224-5p.

Keywords LINC00963; miR-1224-5p; breast cancer; proliferation; radiosensitivity

乳腺癌(breast cancer, BC)是全球范围内威胁女性健康安全的恶性肿瘤之一, 据最新数据显示, 乳腺癌的病发率和死亡率位居所有女性癌症首位, 已经严重影响了患者的生活质量[1]。目前乳腺癌治疗方式仍是以外科手术切除, 辅以结合放疗或化疗为主, 但是患者5年预后生存难以延长、出现耐药性、放疗抵抗等是当前乳腺癌治疗面临的挑战[2-3]。因此, 寻找新的治疗方式对乳腺癌治疗具有重要的意义。长链非编码RNA(long strand non-coding RNA, lncRNA)为长度约为200个核苷酸的非编码RNA, 在多种肿瘤内差异表达, 参与肿瘤恶性进展[4-6]。研究结果显示, 长基因间非编码RNA 00963(long intergene noncoding RNA00963, LINC00963)在乳腺癌中有致癌作用, 沉默LINC00963可抑制乳腺癌细胞增殖, 并增加其放射敏感性[7]。还有研究结果显示, LINC00963高表达与乳腺癌患者的淋巴结转移、TNM分期和分化等有关, 体外实验显示LINC00963沉默能抑制乳腺癌细胞增殖和转移, 并诱导细胞凋亡[8]。在线数据库预测显示, LINC00963与miR-1224-5p有相互结合的位点, miR-1224-5p在卵巢癌、结直肠癌中的表达水平降低, 在肿瘤中有抑癌作用[9-10]。乳腺癌关于miR-1224-5p的研究还不清楚。鉴于此, 本研究以乳腺癌细胞MDA-MB-231为主要研究对象, 探索LINC00963是否可以通过靶向miR-1224-5来调控乳腺癌细胞增殖和放射敏感性。

1 材料方法

1.1 主要试剂

胎牛血清、RPMI1640培养基购于美国Gibco公司; si-NC、si-LINC00963、pcDNA、pcDNA-LINC00963、miR-NC、miR-1224-5p、anti-miR-NC、anti-miR-1224-5p、引物购于上海吉玛生物技术有限公司; Lipofectamine™ 2000试剂盒、TRIzol试剂盒、逆转录试剂盒、AceQ qPCR SYBR® Green Mix试剂盒购于美国Thermo Fisher公司; MTT试剂盒、双荧光素酶报告试剂盒购于北京索莱宝生物技术有限公司; 细胞周期蛋白D1(CyclinD1)抗体、增殖细胞核抗原(proliferating cell nuclear antigen, PCNA)抗体、GAPDH抗体购于北京中山金桥生物技术有限公司。

1.2 细胞培养和分组

乳腺上皮细胞MCF12A、乳腺癌细胞(MDA-MB-231、MDA-MB-468、MCF-7)购于中国科学院上海细胞库。细胞于含10%胎牛血清、青霉素–链霉素混合液的RPMI1640培养基中培养, 随后将其放置在条件为37 °C、5% CO_2的培养箱中。

取对数期MDA-MB-231细胞接种于96孔板内, 当细胞融合度为70%时, 依据脂质体法进行转染, 将si-NC、si-LINC00963、pcDNA、pcDNA-LINC00963、miR-NC、miR-1224-5p、si-LINC00963和anti-miR-NC、si-LINC00963和anti-miR-1224-5p转染至细胞中, 并分别将其命名为si-NC组、si-LINC00963组、pcDNA组、pcDNA-LINC00963组、miR-NC组、miR-1224-5p组、si-LINC00963+anti-miR-NC组、si-LINC00963+anti-miR-1224-5p组。转染步骤参照Lipofectamine™ 2000试剂盒说明书进行。

1.3 qRT-PCR检测LINC00963和miR-1224-5p的相对表达情况

将TRIzol试剂加入乳腺上皮细胞MCF12A、乳腺癌细胞(MDA-MB-231、MDA-MB-468、MCF-7)以及各组培养48 h的MDA-MB-231细胞中, 使用分光光度计检测提取的总RNA, 逆转录合成cDNA第一链, 利用AceQ qPCR SYBR® Green Mix试剂盒进行扩增反应。LINC00963以*GAPDH*作内参、miR-1224-5p以*U6*作内参, 通过$2^{-\Delta\Delta Ct}$法计算其相对表达情

况。引物序列: LINC00963上游5′-GGT AAA TCG AGG CCC AGA GAT-3′, 下游5′-ACG TGG ATG ACA GCG TGT GA-3′; miR-1224-5p上游5′-TGA GGA CTC GGG AGG T-3′, 下游5′-GAA CAT GTC TGC GTA TCT C-3′; *GAPDH*上游5′-GGA GCG AGA TCC CTC CAA AAT-3′, 下游5′-GGC TGT TGT CAT ACT TCT CAT GG-3′; *U6*上游5′-GCT TCG GCA GCA CAT ATA CTA A-3′, 下游5′-AAC GCT TCA CGA ATT TGC GT-3′。

1.4 MTT检测细胞增殖情况

取对数期MDA-MB-231细胞接种于96孔板中, 依据1.2法处理细胞, pcDNA组、pcDNA-LINC00963组除外, 在培养箱中分别培养24 h、48 h、72 h, 每孔内加20 μL的MTT试剂, 继续培养4 h, 加入150 μL DMSO试剂, 轻轻振荡至结晶溶解, 在酶标仪490 nm处检测各孔吸光度(*D*)值。

1.5 Western blot检测CyclinD1、PCNA蛋白表达情况

加入蛋白裂解液至培养48 h的MDA-MB-231细胞(pcDNA组、pcDNA-LINC00963组除外)中, 提取细胞总蛋白, BCA法进行蛋白测量, 每个蛋白质样品上样量为50 μg, 经SDS-PAGE分离, 并电转到PVDF膜上。脱脂奶粉室温封闭培养2 h, CyclinD1(稀释1:600)、PCNA(稀释1:600)、GAPDH(稀释1:1 000)抗体4 °C过夜孵育, 再加入二抗(稀释1:5 000)室温孵育2 h, 加ECL试剂显影, 曝光。用Quantity One软件分析蛋白灰度值。

1.6 克隆实验检测细胞放射敏感性

将各组MDA-MB-231细胞(pcDNA组、pcDNA-LINC00963组除外)接种在6孔板中, 每孔接种1 000个细胞, 并进行不同剂量(0、2、4、6、8 Gy)照射, 源靶距为100 cm, 剂量率2.4 Gy·min^{-1}, 继续培养14天, 使用甲醛、结晶紫室温固定30 min和染色30 min, 在低倍显微镜下观察细胞克隆数。依据软件GraphPad Prism 7.0用单击多靶模型拟合细胞存活曲线, 计算相应参数值。

1.7 双荧光素酶报告实验检测LINC00963和miR-1224-5p靶向关系

双荧光素酶报告实验的本质在于将感兴趣的基因的启动子区域与荧光素酶基因构建成一个融合基因转染至目标细胞(如MDA-MB-231), 检测该基因的转录活性。若将靶向基因的启动子区域与荧光素酶基因融合, 在同一细胞中加入影响目标基因转录的构建物(如siRNA、miRNA), 通过评估siRNA、miRNA等构建物对目标基因转录活性的影响, 或是促进或是抑制目标基因的转录, 继而判断构建物对目标基因的靶向关系。该法具有误差较小、灵敏度和特异性均较高等优点。在线数据库预测显示, LINC00963 3′-UTR与miR-1224-5p存在结合位点。以基因突变技术, 将LINC00963预测结合miR-1224-5p的位点进行突变, 将LINC00963结合miR-1224-5p的突变位点克隆至pGL3质粒, 人工构建突变型载体MUT-LINC00963。将miR-1224-5p结合miR-1224-5p位点以分子克隆法克隆至pGL3质粒, 人工构建野生型载体WT-LINC00963。取对数期MDA-MB-231细胞接种至24孔板中, 待细胞生长融合至80%, 将上述载体以Lipofectamin™ 2000转染试剂分别与miR-NC或miR-1224-5p共转染至细胞, 37 °C孵育24 h, 依据双荧光素酶报告试剂盒说明书步骤, 检测细胞相对荧光素酶活性。

1.8 统计学分析

使用SPSS 22.0软件分析处理数据, 采用Kolmogorov-Smirnov检验本研究所有的计量资料均符合正态分布, 以均值±标准差($\bar{x}\pm s$)表示, 多组间使用单因素方差分析, 多组间的两两比较采用LSD-*t*检验, 两组间比较使用*t*检验。$P<0.05$为差异有统计学意义。

2 结果

2.1 LINC00963和miR-1224-5p在乳腺癌细胞中的表达

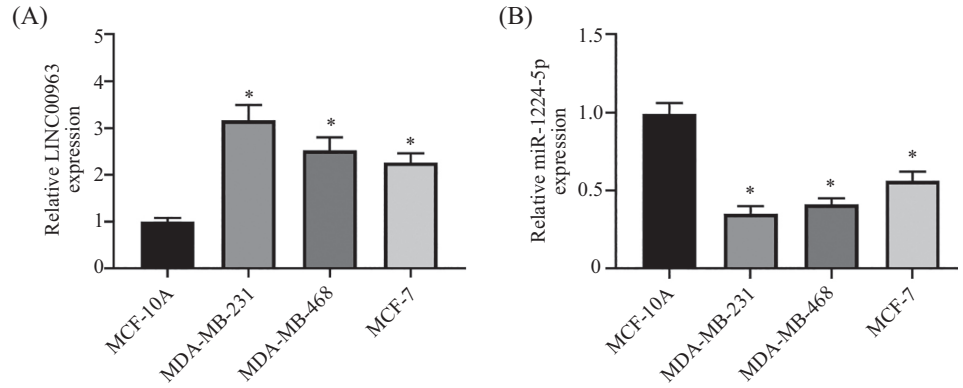
与乳腺上皮细胞MCF-10A相比较, 3种乳腺癌细胞(MDA-MB-231、MDA-MB-468、MCF-7)中LINC00963相对表达水平显著增加($P<0.05$), miR-1224-5p相对表达水平显著降低($P<0.05$)(图1和表1)。

2.2 抑制LINC00963表达对乳腺癌细胞增殖的影响

与si-NC组比较, si-LINC00963组乳腺癌细胞中LINC00963相对表达显著降低($P<0.05$), 细胞*D*值显著降低($P<0.05$), CyclinD1、PCNA蛋白表达量也显著降低($P<0.05$)(图2和表2)。

2.3 抑制LINC00963表达对乳腺癌细胞放射敏感性的影响

随着照射剂量的增加, 与si-NC组相比较, si-LINC00963组的细胞存活分数显著降低($P<0.05$);



A: LINC00963在乳腺癌细胞中的表达; B: miR-1224-5p在乳腺癌细胞中的表达。*P<0.05, 与MCF-10A组比较。

A: LINC00963 expression in breast cancer cells; B: miR-1224-5p expression in breast cancer cells. *P<0.05 compared with MCF-10A group.

图1 LINC00963和miR-1224-5p在乳腺癌细胞中的表达

Fig.1 LINC00963 and miR-1224-5p expression in breast cancer cells

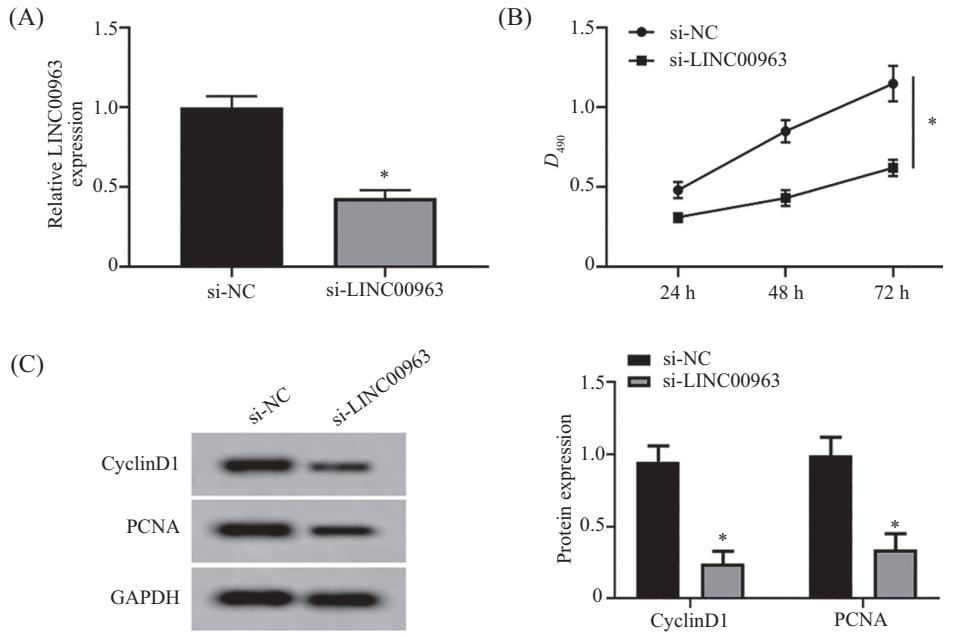
表1 LINC00963和miR-1224-5p在乳腺癌细胞中的表达

Table 1 Expression of LINC00963 and miR-1224-5p in breast cancer cells

分组 Groups	LINC00963	miR-1224-5p
MCF-10A	1.00±0.08	0.99±0.07
MDA-MB-231	3.17±0.32*	0.35±0.05*
MDA-MB-468	2.52±0.28*	0.41±0.04*
MCF-7	2.26±0.20*	0.56±0.06*
F	131.053	238.357
P	0.000	0.000

$\bar{x}\pm s$; n=9; *P<0.05, 与MCF-10A组比较。

$\bar{x}\pm s$; n=9; *P<0.05 compared with MCF-10A group.



A: si-LINC00963转染效率的检测; B: 干扰LINC00963对乳腺癌细胞增殖的影响; C: CyclinD1、PCNA蛋白表达情况。*P<0.05, 与si-NC组比较。

A: the transfection efficiency of interfering LINC00963; B: effect of interfering LINC00963 on the proliferation of breast cancer cells; C: CyclinD1, PCNA protein expression. *P<0.05 compared with si-NC group.

图2 抑制LINC00963表达对乳腺癌细胞增殖的影响

Fig.2 Effect of LINC00963 knockdown on breast cancer cell proliferation

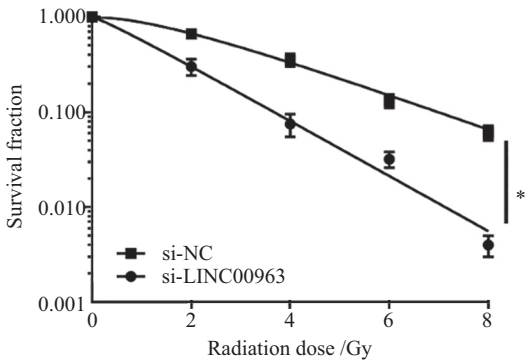
表2 抑制LINC00963对乳腺癌细胞增殖的影响

Table 2 Effect of inhibition of LINC00963 on breast cancer cell proliferation

分组 Groups	LINC00963	D_{490}			CyclinD1	PCNA
		24 h	48 h	72 h		
si-NC	1.00±0.07	0.48±0.05	0.85±0.07	1.15±0.11	0.95±0.11	0.99±0.13
si-LINC00963	0.43±0.05*	0.31±0.03*	0.43±0.05*	0.62±0.05*	0.24±0.09*	0.34±0.11*
t	19.878	8.746	14.647	13.159	14.987	11.451
P	0.000	0.000	0.000	0.000	0.000	0.000

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

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



*P<0.05, 与si-NC组比较。

*P<0.05 compared with si-NC group.

图3 抑制LINC00963表达对乳腺癌细胞存活分数的影响

Fig.3 Effect of inhibition of LINC00963 expression on the survival fraction of breast cancer cells

表3 单击多靶模型参数

Table 3 Click on multi-target model parameters

分组 Groups	D_0 /Gy	D_q /Gy	N	SF_2	k	SER
si-NC	2.353	1.627	1.997	0.672	0.425	
si-LINC00963	1.497	0.241	1.175	0.301	0.668	1.572

si-NC组中D_0、D_q、SF_2(survival fraction at 2 Gy)为2.353、1.627、0.672, si-LINC00963组D_0、D_q、SF_2为1.497、0.247、0.301, 其中放射增敏比(sensitizing enhancement ratio, SER)为1.572(图3和表3)。

2.4 LINC00963靶向调控miR-1224-5p的表达

LINC00963的序列中含有与miR-1224-5p互补的核苷酸序列。与miR-NC组相比较, miR-1224-5p组中WT-LINC00963荧光素酶活性显著降低(P<0.05), 而miR-1224-5p组中MUT-LINC00963荧光素酶活性没有显著变化。与pcDNA组比较, pcDNA-LINC00963组中miR-1224-5p相对表达水平显著降低(P<0.05); 与si-NC组比较, si-LINC00963组中miR-1224-5p相对表达水平显著增加(P<0.05)(图4、图5、图6、表4和表5)。

2.5 过表达miR-1224-5p对乳腺癌细胞增殖和放射敏感性的影响

与miR-NC组相比较, miR-1224-5p组乳腺癌细胞中miR-1224-5p相对表达水平显著增加(P<0.05), 细胞D值显著降低(P<0.05), CyclinD1、PCNA蛋白表达水平也显著降低(P<0.05)(图7和表6)。

随着照射剂量的增加, 细胞存活分数显著降低(P<0.05), 其中miR-NC组高于miR-1224-5p组; miR-NC组中D_0、D_q、SF_2为2.141、1.736、0.675, miR-1224-5p组D_0、D_q、SF_2为1.355、0.604、0.333, 其中放射增敏比为1.580(图8和表7)。

2.6 干扰miR-1224-5p逆转抑制LINC00963表达对乳腺癌细胞增殖和放射敏感性的影响

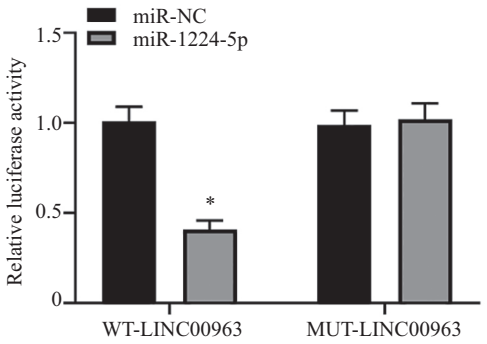
与si-LINC00963+anti-miR-NC组比较, si-

WT-LINC00963 5′ guGCCU−UAGUGUCCUCAu 3′
miR-1224-5p 3′ ggUGGAGGGCUCAGGAGUg 5′

MUT-LINC00963 5′ guGGGA−UACUCAGGAGUu 3′

图4 LINC00963的序列中含有与miR-1224-5p互补的核苷酸序列

Fig.4 The sequence of LINC00963 contains a nucleotide sequence complementary to miR-1224-5p

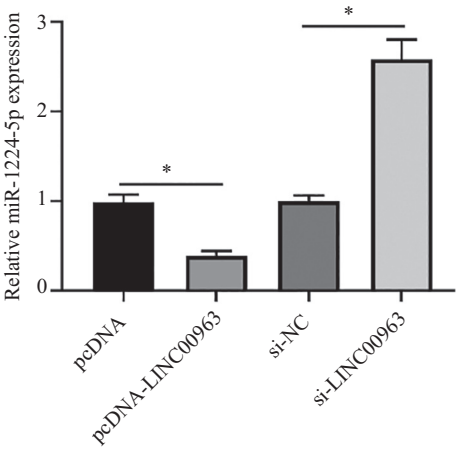


*P<0.05, 与miR-NC组比较。

*P<0.05 compared with miR-NC group.

图5 双荧光素酶报告实验

Fig.5 Dual luciferase reporter experiment



*P<0.05.

图6 LINC00963调控miR-1224-5p表达

Fig.6 LINC00963 regulates miR-1224-5p expression

表4 双荧光素酶报告实验

Table 4 Dual luciferase reporter experiment

分组 Groups	WT-LINC00963	MUT-LINC00963
miR-NC	1.01±0.08	0.99±0.08
miR-1224-5p	0.41±0.05*	1.02±0.09
t	19.080	0.747
P	0.000	0.466

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

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

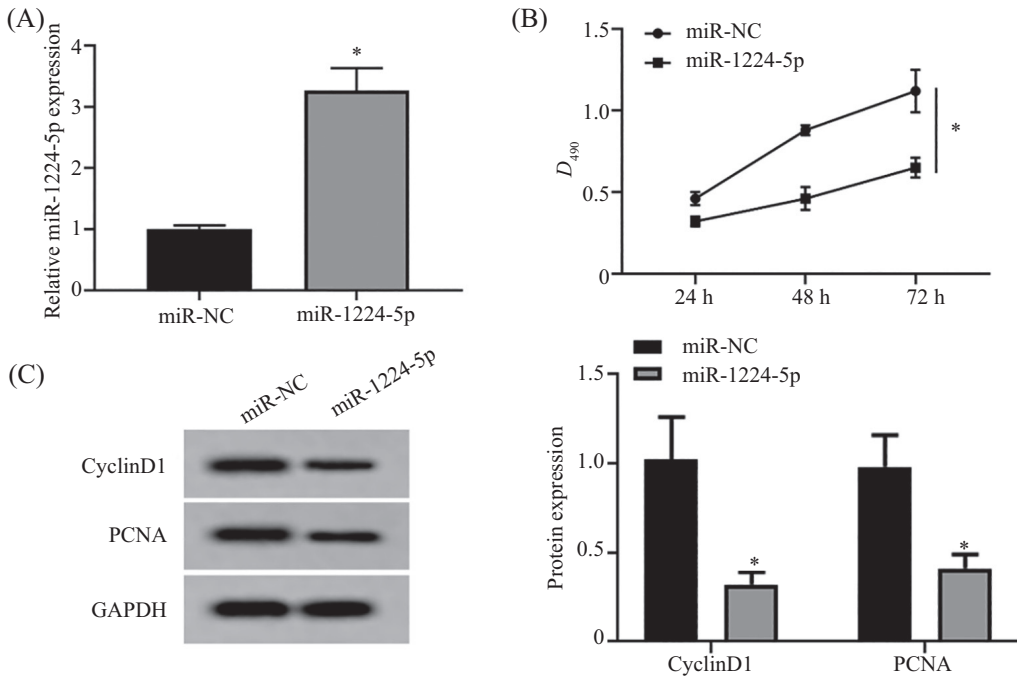
表5 LINC00963调控miR-1224-5p表达

Table 5 LINC00963 regulates miR-1224-5p expression

分组 Group	miR-1224-5p
pcDNA	0.99±0.08
pcDNA-LINC00963	0.39±0.05*
si-NC	1.00±0.06
si-LINC00963	2.58±0.22[%]
F	519.842
P	0.000

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

$\bar{x}\pm s$; n=9; *P<0.05 compared with pcDNA group; [%]P<0.05 compared with si-NC group.



A: miR-1224-5p过表达效率的检测; B: 过表达miR-1224-5p对乳腺癌细胞增殖的影响; C: CyclinD1、PCNA蛋白表达情况。*P<0.05, 与miR-NC组比较。

A: transfection efficiency of overexpressing miR-1224-5p; B: effect of miR-1224-5p overexpression on breast cancer cell proliferation; C: CyclinD1, PCNA protein expression. *P<0.05 compared with miR-NC group.

图7 过表达miR-1224-5p对乳腺癌细胞增殖的影响

Fig.7 Effect of overexpression of miR-1224-5p on proliferation of breast cancer cells

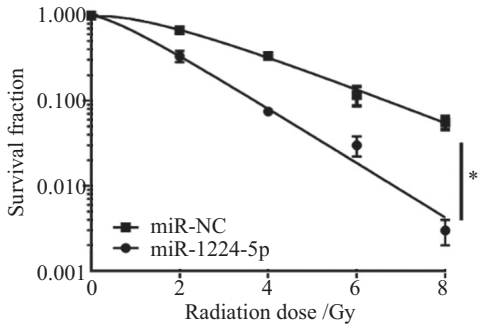
表6 过表达miR-1224-5p对乳腺癌细胞增殖的影响

Table 6 Effect of overexpression of miR-1224-5p on proliferation of breast cancer cell

分组 Groups	miR-1224-5p	D_{450}			CyclinD1	PCNA
		24 h	48 h	72 h		
miR-NC	1.00±0.06	0.46±0.04	0.88±0.08	1.12±0.13	1.02±0.24	0.98±0.18
miR-1224-5p	3.27±0.36*	0.32±0.03*	0.46±0.07*	0.65±0.06*	0.32±0.07*	0.41±0.08*
t	18.659	8.400	11.853	9.848	8.400	8.681
P	0.000	0.000	0.000	0.000	0.000	0.000

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

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



*P<0.05.

图8 过表达miR-1224-5p对乳腺癌细胞存活分数的影响

Fig.8 Effect of overexpression of miR-1224-5p on the survival fraction of breast cancer cells

表7 单击多靶模型参数

Table 7 Parameters of the one-click multi-target model

分组 Groups	D_0 /Gy	D_q /Gy	N	SF_2	k	SER
miR-NC	2.141	1.736	2.250	0.675	0.467	
miR-1224-5p	1.355	0.604	1.562	0.333	0.738	1.580

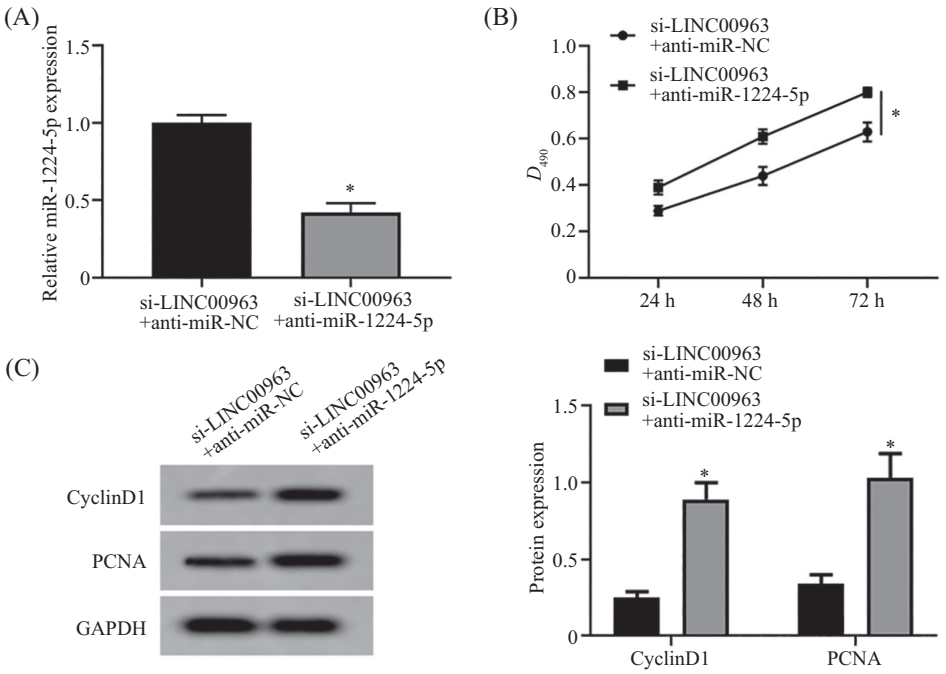
LINC00963+anti-miR-1224-5p组乳腺癌细胞中miR-1224-5p相对表达显著降低(P<0.05), 细胞D值显著增加(P<0.05), CyclinD1、PCNA蛋白表达水平也显著增加(P<0.05)(图9和表8)。

随着照射剂量的增加, 与si-LINC00963+anti-miR-NC相比较, si-LINC00963+anti-miR-1224-5p组的细胞存活分数显著降低(P<0.05)。si-LINC00963+anti-miR-NC组中D_0、D_q、SF_2为1.435、0.471、0.327, si-LINC00963+anti-miR-1224-5p组D_0、D_q、SF_2为1.869、1.136、0.538, 其中放射增敏比为0.768(图10和表9)。

3 讨论

乳腺癌治疗方式中放射治疗应用较为广泛, 在一定程度上能延长乳腺癌患者生存期限, 但是还有部分患者出现放疗抵抗性[11]。近年的研究结果显示, lncRNA与肿瘤放疗敏感密切相关[12], 如lncRNA PCAT6在三阴性乳腺癌组织中表达上调, miR-185-5p是直接靶基因, 敲除lncRNA PCAT6通过上调miR-185-5p增加三阴性乳腺癌细胞放射敏感性[13]。乳腺癌细胞中致癌基因lncRNA HOTAIR可能通过靶向抑制miR-449b-5p增加乳腺癌细胞放射敏感性[14]。在乳腺癌组织中LINC00511表达量增加, 敲低LINC00511能抑制细胞增殖和诱导细胞凋亡, 并增加细胞放射敏感性[15]。还有研究结果显示, 乳腺癌细胞lncRNA TUG1高表达, 可能通过靶向抑制miR-214-5p增加乳腺癌细胞放射敏感性[16]。LINC00963在乳腺癌细胞中表达量增加, 与不良预后有关, 且与乳腺癌细胞增殖、凋亡及放射敏感性等有关[7-8,17]。本研究使用qRT-PCR检测乳腺上皮细胞MCF-10A和乳腺癌细胞(MDA-MB-231、MDA-MB-468、MCF-7)中LINC00963的表达情况, 并检测抑制LINC00963对乳腺癌细胞增殖和放射敏感性的影响。本研究结果中显示, LINC00963在乳腺癌细胞中相对表达水平高于乳腺上皮细胞, 抑制LINC00963降低乳腺癌细胞增殖活性和细胞存活分数, 且下调CyclinD1、PCNA蛋白表达, 提示抑制LINC00963能够抑制乳腺癌细胞增殖并增加乳腺癌细胞放射敏感性。

胶质瘤细胞中miR-1224-5p低表达, 与乳腺癌患者病理分级有关, 上调miR-1224-5p能有效抑制胶质瘤细胞增殖[18]。结直肠癌细胞中miR-1224-5p低表达, *SLC29A3*是miR-1224-5p的靶基因, miR-1224-5p通过靶向调控SLC29A3抑制结直肠癌细胞增殖、迁移和侵袭[19]。miR-1224-5p在骨肉瘤组织和细胞中低表达, 过表达miR-1224-5p能有效抑制骨肉路细胞增殖和侵袭, 并诱导细胞凋亡[20]。研究结果显示, 食管鳞状细胞癌组织和细胞中miR-1224-5p表达量降低, 其低表达与患者较短的生存期限有关, miR-1224-5p可能通过靶向抑制TNS4/EGFR轴进而抑制食管鳞状细



A: 抑制LINC00963和干扰miR-1224-5p对miR-1224-5p表达的影响; B: 抑制LINC00963和干扰miR-1224-5p对增殖的影响; C: CyclinD1、PCNA蛋白表达情况。*P<0.05, 与si-LINC00963+anti-miR-NC组比。

A: effect of LINC00963 knockdown and miR-1224-5p inhibitor on miR-1224-5p expression; B: effect of LINC00963 downregulation and miR-1224-5p inhibitor on breast cancer cell proliferation; C: CyclinD1, PCNA protein expression. *P<0.05 compared with si-LINC00963+anti-miR-NC group.

图9 干扰miR-1224-5p逆转抑制LINC00963表达对乳腺癌细胞增殖的影响

Fig.9 Interference with miR-1224-5p reversed LINC00963 depletion-mediated effect on breast cancer cell proliferation

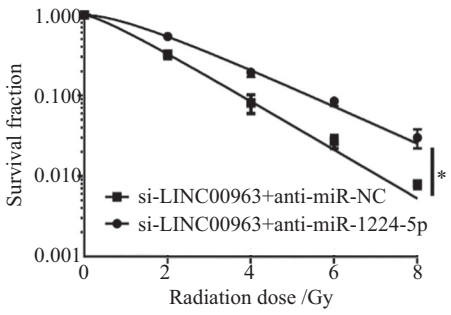
表8 干扰miR-1224-5p逆转抑制LINC00963表达对乳腺癌细胞增殖的影响

Table 8 Effect of interfering with miR-1224-5p to reverse the inhibition of LINC00963 expression on breast cancer cell proliferation

分组 Groups	miR-1224-5p	D_{450}			CyclinD1	PCNA
		24 h	48 h	72 h		
si-LINC00963+anti-miR-NC	1.00±0.05	0.29±0.02	0.44±0.04	0.63±0.04	0.25±0.04	0.34±0.06
si-LINC00963+anti-miR-1224-5p	0.42±0.06*	0.39±0.03*	0.61±0.03*	0.80±0.02*	0.89±0.11*	1.03±0.16*
t	22.278	8.321	10.200	11.404	16.404	12.114
P	0.000	0.000	0.000	0.000	0.000	0.000

$\bar{x}\pm s$; n=9; *P<0.05, 与si-LINC00963+anti-miR-NC组比较。

$\bar{x}\pm s$; n=9; *P<0.05 compared with si-LINC00963+anti-miR-NC group.



*P<0.05.

图10 干扰miR-1224-5p表达逆转了抑制LINC00963表达对乳腺癌细胞存活分数的影响

Fig.10 Interfering with miR-1224-5p expression reverses the effect of inhibiting LINC00963 expression on the survival fraction of breast cancer cells

表9 单击多靶模型参数

Table 9 Click on multi-target model parameters

分组 Groups	D_0 /Gy	D_q /Gy	N	SF_2	k	SER
si-LINC00963+anti-miR-NC	1.435	0.471	1.389	0.327	0.697	
si-LINC00963+anti-miR-1224-5p	1.869	1.136	1.836	0.538	0.535	0.768

胞癌细胞增殖、迁移和侵袭[21]。但miR-1224-5p对乳腺癌细胞的调控作用及机制尚不明确。本研究结果显示, 与乳腺上皮细胞相比, 乳腺癌细胞中miR-1224-5p表达水平降低, 过表达miR-1224-5p降低乳腺癌细胞增殖活性和细胞存活分数, 且下调CyclinD1、PCNA蛋白表达水平, 提示过表达miR-1224-5p抑制乳腺癌细胞增殖, 并增加细胞放射敏感性。在线生物信息软件和双荧光素酶报告实验显示, LINC00963可靶向调控miR-1224-5p。上调LINC00963降低miR-1224-5p相对表达量, 抑制LINC00963增加miR-1224-5p相对表达量, 说明LINC00963可靶向抑制miR-1224-5p, 进一步实验结果显示, 干扰miR-1224-5p逆转了抑制LINC00963表达对乳腺癌细胞增殖和放射敏感性的作用, 提示LINC00963可靶向抑制miR-1224-5p影响乳腺癌进展。

综上所述, 乳腺癌细胞中LINC00963高表达, miR-1224-5p低表达, 抑制LINC00963可有效抑制乳腺癌细胞增殖, 提高乳腺癌细胞放射敏感性, 并靶向抑制miR-1224-5p。

参考文献 (References)

[1] KATSURA C, OGUNMWONYI I, KANKAM H K, et al. Breast cancer: presentation, investigation and management [J]. Br J Hosp Med, 2022, 83(2): 1-7.

[2] KASHYAP D, PAL D, SHARMA R, et al. Global increase in breast cancer incidence: risk factors and preventive measures [J]. Biomed Res Int, 2022, 2022(4): 9605439-60.

[3] CASTANEDA S A, STRASSER J. Updates in the treatment of breast cancer with radiotherapy [J]. Surg Oncol Clin N Am, 2017, 26(3): 371-82.

[4] WINKLER L, DIMITROVA N. A mechanistic view of long noncoding RNAs in cancer [J]. Wiley Interdiscip Rev RNA, 2022, 13(3): e1699-725.

[5] BHAN A, SOLEIMANI M, MANDAL S S. Long noncoding RNA and cancer: a new paradigm [J]. Cancer Res, 2017, 77(15): 3965-81.

[6] TAN Y T, LIN J F, LI T, et al. LncRNA-mediated posttranslational modifications and reprogramming of energy metabolism in cancer [J]. Cancer Commun, 2021, 41(2): 109-20.

[7] ZHANG N, ZENG X, SUN C, et al. LncRNA LINC00963 promotes tumorigenesis and radioresistance in breast cancer by sponging miR-324-3p and inducing ACK1 expression [J]. Mol Ther Nucleic Acids, 2019, 18(12): 871-81.

[8] WU Z, WANG W, WANG Y, et al. Long noncoding RNA LINC00963 promotes breast cancer progression by functioning as a molecular sponge for microRNA-625 and thereby upregulating HMGA1 [J]. Cell Cycle, 2020, 19(5): 610-24.

[9] WANG J, HU Y, YE C, et al. miR-1224-5p inhibits the proliferation and invasion of ovarian cancer via targeting SND1 [J]. Hum Cell, 2020, 33(3): 780-9.

[10] GUO T, PENG S, LIU D, et al. Long non-coding RNA LOXL1-AS1 facilitates colorectal cancer progression via regulating miR-1224-5p/miR-761/HK2 axis [J]. Biochem Genet, 2022, 60(6): 2416-33.

[11] HAUSSMANN J, CORRADINI S, NESTLE-KRAEMLING C, et al. Recent advances in radiotherapy of breast cancer [J]. Radiat Oncol, 2020, 15(1): 71-83.

[12] YAO Z, ZHANG Y, XU D, et al. Research progress on long non-coding RNA and radiotherapy [J]. Med Sci Monit, 2019, 25: 5757-70.

[13] SHI R, WU P, LIU M, et al. Knockdown of lncRNA PCAT6 enhances radiosensitivity in triple-negative breast cancer cells by regulating miR-185-5p/TPD52 axis [J]. Onco Targets Ther, 2020, 13: 3025-37.

[14] ZHANG S, WANG B, XIAO H, et al. LncRNA HOTAIR enhances breast cancer radioresistance through facilitating HSPA1A expression via sequestering miR-449b-5p [J]. Thorac Cancer, 2020, 11(7): 1801-16.

[15] LIU L, ZHU Y, LIU A M, et al. Long noncoding RNA LINC00511 involves in breast cancer recurrence and radioresistance by regulating STXBP4 expression via miR-185 [J]. Eur Rev Med Pharmacol Sci, 2019, 23(17): 7457-68.

[16] 孙露阳, 葛锁华, 彭俊华, 等. 沉默LncRNA TUG1通过上调miR-214-5p表达增加乳腺癌细胞的放射敏感性[J]. 中国免疫学杂志(SUN L Y, GE S H, PENG J H, et al. Silencing LncRNA TUG1 increases radiosensitivity of breast cancer cells by upregulating expression of miR-214-5p [J]. Chinese Journal of Immunology), 2021, 37(16): 1984-90.

[17] XIONG H, CHEN Z, CHEN W, et al. FKBP-related ncRNA-mRNA axis in breast cancer [J]. Genomics, 2020, 112(6): 4595-607.

[18] 钱进, 徐英纳, 栾文康, 等. miR-1224-5p在胶质瘤中的表达及其对胶质瘤细胞增殖的影响[J]. 临床神经外科杂志(QIAN J, XU Y N, LUAN W K, et al. Expression of miR-1224-5p in brain glioma and its role in proliferation of glioma cells [J]. Journal of Clinical Neurosurgery), 2016, 13(5): 325-7,31.

[19] SONG N S, PEI Z D, FU G. MiR-1224-5p acts as a tumor suppressor via inhibiting the malignancy of rectal cancer through targeting SLC29A3 [J]. IUBMB Life, 2020, 72(10): 2204-13.

[20] JIN B, JIN D, ZHUO Z, et al. MiR-1224-5p activates autophagy, cell invasion and inhibits epithelial-to-mesenchymal transition in osteosarcoma cells by directly targeting PLK1 through PI3K/AKT/mTOR signaling pathway [J]. Onco Targets Ther, 2020, 13: 11807-18.

[21] SHI Z Z, WANG W J, CHEN Y X, et al. The miR-1224-5p/TNS4/EGFR axis inhibits tumour progression in oesophageal squamous cell carcinoma [J]. Cell Death Dis, 2020, 11(7): 597-606.