DOI:10.3872/j.issn.1007-385x.2020.12.003

·基础研究·

miR-9-5p对乳腺癌恶性生物学行为的影响及其调控机制

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[摘 要] **目的**:探讨miR-9-5p在乳腺癌恶性生物学行为中发挥的作用及其可能的调控机制。**方法**:利用OncomiR在线数据库分析miR-9-5p在乳腺癌组织与正常乳腺组织中表达的差异,qPCR检测乳腺癌细胞系与正常乳腺细胞中miR-9-5p表达水平。基于靶基因预测软件TargetScan分析ONECUT2(one cut homeobox 2)可能是miR-9-5p的作用靶基因,双荧光素酶报告实验验证两者的靶向关系。向MDA-231细胞中分别转染miR-9-5p mimic、ONECUT2 siRNA及相应对照,qPCR及WB实验检测转染对MDA-231细胞中干性基因NOTCH1、NANOG和Y染色体性别决定区(sex-determing region of Y chromosome,SRY)-盒转录因子9(SRY-box transcription factor 9,SOX9)表达水平的影响,BrdU法、Annexin Ⅴ流式细胞术、MTS实验分别检测转染对细胞增殖、凋亡和化疗耐药的影响,ALDEFLUOR染色流式细胞术检测miR-9-5p及靶基因ONECUT2对肿瘤干细胞化特征的影响。建立NSG小鼠乳腺癌化疗模型,体内实验进一步验证ONECUT2对肿瘤干性化及化疗抵抗等肿瘤恶性生物学行为的影响。**结果**:miR-9-5p在乳腺癌组织(P=0.007)及乳腺癌MDA-231细胞系(P=0.0005)中呈现显著高表达,并与乳腺癌患者不良预后呈正相关(P=0.0016)。miR-9-5p可靶向负调控ONECUT2,进而增加$ALDH^+$MDA-231细胞比例(P=0.0006),上调干性NOTCH1、NANOG和SOX9蛋白表达,并增强乳腺癌细胞抗凋亡能力(P=0.0003)及其对多西他赛(DTX)和多柔比星(DOXO)化疗的耐受性;然而miR-9-5p/ONECUT2轴未能显著影响MDA-231细胞的增殖能力(P>0.05)。与对照组相比,MDA-231/ONECUT2组小鼠接受DTX治疗后,移植瘤体积较对照组显著缩小(P<0.05),瘤组织中NOTCH1、SOX9蛋白和ABC转运蛋白的mRNA和蛋白表达水平均显著降低(P<0.05或P<0.01)。**结论**:乳腺癌组织中高表达的miR-9-5p通过靶向ONECUT2诱导乳腺癌干细胞化及抗凋亡能力,增强了其对化学治疗的抵抗性。

[关键词] 乳腺癌;MDA-231细胞;miR-9-5p;ONECUT2;生物学行为;肿瘤干性;细胞凋亡;化疗抵抗
[中图分类号] R730.5; R737.9 [文献标识码] A [文章编号] 1007-385X(2020)12-1328-08

Regulatory effect and mechanism of miR-9-5p on malignant biological behaviors of breast cancer

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[Abstract] Objective: To explore the role of miR-9-5p in the biological behaviors of breast cancer cells and its possible regulatory mechanism. **Methods:** online OncomiR database was used to analyze the differential expression of miR-9-5p in breast cancer tissues and normal breast tissues. qPCR was used to detect the miR-9-5p expression in breast cancer cell lines and normal breast cells. Based on target gene prediction software TargetScan, ONECUT2 (one cut homeobox 2) was predicted to be the target gene of miR-9-5p. Dual luciferase reporter system was used to validate the relationship between miR-9-5p and its promising target gene ONECUT2. MDA-231 cells were transfected with miR-9-5p mimic, ONECUT2 siRNAs as well as the corresponding control sequences. The protein and mRNA levels of stemness-associated gene NOTCH1, NANOG and SOX9 (SRY (sex-determing region of Y chromosome) -Box transcription Factor 9) were detected by WB and qPCR. The effect of transfection on proliferation, apoptosis and chemo-resistance of cells was detected by BrdU method, Annexin Ⅴ method and MTS Assay, respectively. The ALDEFLUOR experiment was used to detect the effects of miR-9-5p and its target

[基金项目] 天津市教委科研计划资助项目(No. 2019KJ185)。Project supported by the Scientific Research Program of Education Commission of Tianjin (No. 2019KJ185)

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gene ONECUT2 on tumor stemness. NSG mouse breast cancer chemotherapy model was established, and the in vivo experiments further verified the effect of ONECUT2 on tumor malignant biological behaviors, such as cell stemness and chemo-resistance. **Results:** miR-9-5p was highly expressed in breast cancer tissues (P=0.007) and breast cancer MDA-231 cell line (P=0.0005), and was positively correlated with the poor prognosis of breast cancer patients (P=0.0016). Compared to control group, miR-9-5p could target and negatively regulate ONECUT2 expression, further increase $ALDH^+$ cell population (P=0.0006), as well as increase the expressions of stemness-associated genes NOTCH1, NANOG and SOX9. Besides, miR-9-5p increased the anti-apoptosis ability (P=0.0003) and chemo-resistance of MDA-231 cells; however, miR-9-5p/ONECUT2 exerted no significant effect on the proliferation ability of MDA-231 cells (P>0.05). Compared with the control group, the volume of xenografts in mice of MDA-231/ONECUT2 group after DTX chemotherapy was significantly lower than that in the control group (P<0.05), and the protein expressions of NOTCH1, SOX9 and the mRNA expression of ABC transporter in the transplanted tumor tissues were significantly reduced (P<0.05 or P<0.01). **Conclusions:** The highly expressed miR-9-5p in breast cancer induces tumor stemness and anti-apoptotic ability by targeting ONECUT2 and enhances its resistance to chemotherapy.

[Key words] breast cancer; MDA-231 cell; miR-9-5p; ONECUT2; biological behavior; cancer stemness; apoptosis; chemotherapy resistance

[Chin J Cancer Biother, 2020, 27(12): 1328-1335. DOI：10.3872/j.issn.1007-385X.2020.12.003]

乳腺癌居现代女性癌症发生率的首位，对女性健康造成严重影响。乳腺癌发病机制复杂，多种致病因素参与调控乳腺癌的发生和发展，探究乳腺癌的发病机制对指导临床治疗意义重大。miRNA是具有内源性基因调控作用的非编码RNA，能够通过互补配对的方式结合靶基因mRNA的3′UTR区，参与靶基因的转录后调控，进而影响肿瘤恶性生物学行为。miR-9-5p在NSCLC[1]、前列腺癌[2]及乳腺癌[3]等肿瘤中高表达，与肿瘤细胞的血管生成、转移、EMT及干性化[4-6]等生物学行为密切相关。然而有关miR-9-5p如何参与调控乳腺癌肿瘤微环境及其具体机制少有研究。本研究旨在阐明miR-9-5p在乳腺癌组织中的表达水平及其在乳腺癌细胞MDA-231细胞中通过靶向调控ONECUT2（one cut homeobox 2）基因对肿瘤干细胞化特征、凋亡能力和化疗药物耐受性的影响。

1 材料与方法

1.1 主要试剂和仪器

MDA-231、MCF-7、BT474和MCF10A乳腺癌细胞均购自ATCC公司，pCMV-6空白质粒和ONECUT2过表达质粒pCMV-ONECUT2均购自ORIGENE公司，ONECUT2 siRNA[SI04346384（#1），SI04340049（#2）]、对照siRNA（Neg siRNA）、miR-9-5p mimic、对照miRNA mimic（Neg miRNA mimic）均购自QIAGEN公司。PCR引物由上海生工生物工程有限公司合成。非肥胖糖尿病/重症联合免疫缺陷/IL-2Rγ基因敲除（NOD/SCID/IL2Rγ-null，NSG）小鼠购自斯贝福生物技术有限公司[动物合格证号：SCXK（京）2019-0010]。荧光素酶检测试剂盒和MTS试剂盒均购自Promega公司，ALDEFLUOR Assay试剂盒购自Stemcell Technologies公司，Annexin Ⅴ和BrdU试剂盒购自BD公司，Lipofectamine2000和RNAiMax转染试剂购自Thermo Fisher公司，RNA及miRNA反转录试剂盒购自Applied Biosystems公司，ONECUT2和NANOG抗体购自Proteintech公司，NOTCH1、Y染色体性别决定区（sex-determing region of Y chromosome，SRY）-盒转录因子9（SRY-box transcription factor 9，SOX9）及GAPDH等蛋白质的一抗抗体购自Cell Signaling Technology公司，HRP标记山羊抗兔IgG购自中杉金桥公司，全自动酶标仪购自Thermo Scientific公司，7500 qPCR系统购自Applied Biosystems公司，FACS流式细胞仪购自BD公司。

1.2 向MDA-231细胞转染miR-9-5p mimic和ONECUT2 siRNA

取对数生长期MDA-231细胞，将其分为4个转染组，分别转染miR-9-5p mimic、ONECUT2 siRNA以及各自的对照Neg miRNA mimic和Neg siRNA组和仅加入等体积PBS的MDA-231细胞的空白对照组。分别按转染试剂Lipofectamine2000及RNAiMax说明书进行操作，48 h后收集细胞，检验转染效率。

1.3 筛选建立稳定过表达ONECUT2基因的细胞系MDA-231/ONECUT2

MDA-231细胞转染pCMV-ONECUT2过表达质粒，24 h后更换无抗生素完全培养基，同时加入G418（新霉素抗性）至终质量浓度6.4 mg/ml，每日更换含G418培养基，直至细胞正常生长；以仅加入等体积PBS的MDA-231细胞为空白对照组。应用WB实验验证ONECUT2蛋白质在MDA-231/ONECUT2细胞系中的表达水平。

1.4 ALDEFLUOR染色流式术检测miR-9-5p和ONECUT2表达对$ALDH^+$MDA-231细胞比例的影响

取各组经处理的MDA-231细胞1×10^6个，按照ALDEFLUOR Assay试剂盒说明书，实验管加入经活化ALDEFLUOR试剂5 μl，对照管同时加入DEAB，37 ℃孵育45 min，洗涤后采用FACS技术检测$ALDH^+$细胞的比例。

1.5　qPCR检测MDA-231细胞中相关基因的表达水平

取各组经处理的MDA-231细胞1×10⁶个，采用TRIzol法提取RNA，逆转录成cDNA，分别加入mRNA特异性引物（引物序列见表1），应用qPCR法扩增片段，检测ONECUT2、NOTCH1、NANOG、SOX9和腺苷三磷酸结合盒转运蛋白B1、C1、G2和B5（ATP-binding cassette transporter protein B1, C1, G2 and B5, ABCB1，ABCC1，ABCG2和ABCB5）基因的表达水平。以18S rRNA作为内参，用$2^{-\triangle\triangle CT}$法计算基因表达量。

表1 qPCR引物序列

Tab.1 Sequences of primers used in qPCR

Target gene	Sequence
ONECUT2	F: CAAACGCCCGTCAAAGGAGAT
	R: GCTCAGATCGTCTTGCCACTT
NOTCH1	F: GAGGCGTGGCAGACTATGC
	R: CTTGTACTCCGTCAGCGTGA
NANOG	F: CCCCAGCCTTTACTCTTCCTA
	R: CCAGGTTGAATTGTTCCAGGTC
SOX9	F: AGCGAACGCACATCAAGAC
	R: CTGTAGGCGATCTGTTGGGG
18S rRNA	F: CTACCACATCCAAGGAAGGCA
	R: TTTTTCGTCACTACCTCCCCG
ABCB1	F: GCCTGGCAGCTGGAAGACAAATAC
	R: ATGGCCAAAATCACAAGGGTTAGC
ABCC1	F: AGTGGAACCCCTCTCTGTTTAAG
	R: CCTGATACGTCTTGGTCTTCATC
ABCG2	F: CAGGTGGAGGCAAATCTTCGT
	R: ACACACCACGGATAAACTGA
ABCB5	F: CACAAAAGGCCATTCAGGCT
	R: GCTGAGGAATCCACCCAATCT

1.6　WB检测miR-9-5p mimic和ONECUT2 siRNA转染对MDA-231细胞中ONECUT2、NOTCH1、NANOG及SOX9蛋白表达的影响

取经处理的各组MDA-231细胞，裂解细胞、抽提总蛋白，BCA法定量，取蛋白样品行SDS-PAGE，转膜，BSA封闭，分别加入ONECUT2（1∶500）、NANOG（1∶1 000）、NOTCH1（1∶1 000）、SOX9（1∶1 000）和GAPDH（1∶1 000）一抗孵育过夜，清洗后孵育二抗（HRP标记山羊抗兔IgG，1∶4 000），清洗后显影、曝光。利用Image J 1.49软件分析蛋白条带灰度值，蛋白相对表达量为目的蛋白与内参蛋白（GAPDH）条带灰度值的比值。

1.7　Annexin Ⅴ流式术检测miR-9-5p和ONECUT2对MDA-231细胞凋亡的影响

取各组经处理的MDA-231细胞1×10⁶个，洗涤后加入5 μl Annexin Ⅴ和5 μl PI试剂，避光孵育15 min，1 h内采用FACS技术检测凋亡细胞的比例。

1.8　BrdU掺入法检测miR-9-5p和ONECUT2表达对MDA-231细胞增殖的影响

取各组经处理的MDA-231细胞1×10⁶个，按BrdU细胞试剂盒说明书操作，共培养结束前12 h行BrdU掺入，FACS技术检测增殖细胞比例。

1.9　MTS实验检测miR-9-5p mimic对MDA-231细胞化疗耐药性的影响

取各组经处理的MDA-231细胞接种于96孔板中，分别用多西他赛（DTX）10 nmol/L及多柔比星（DOXO）500 nmol/L处理48 h后，每孔加入20 μl MTS/PMS试剂，37 ℃处理1.5 h，酶标仪检测490 nm波长处各孔光密度（D）值。细胞相对活力=$D_{实验组}/D_{PBS}$。

1.10　MDA-231 NSG小鼠化疗模型分析ONECUT2对肿瘤干细胞化及化疗抵抗的影响

选取7~8周龄NSG雌性小鼠8只，随机分为2组，每组4只。将MDA-231和MDA-231/ONECUT2细胞按2×10⁵个/只分别接种于第4乳腺脂肪垫双侧，待肿瘤生长至约300 mm³后，外科手术摘取左侧肿瘤并无菌缝合。按小鼠体质量计算出DTX注射剂量为15 mg/kg，每周一次，共注射3次。治疗结束后用颈椎脱臼法处死小鼠，取右侧肿瘤组织备用。将2组化疗前后的肿瘤组织置于全自动组织匀浆机中，制备RNA及蛋白样品，WB检测ONECUT2、NOTCH1、NANOG、SOX9和GAPDH的表达，qPCR检测ABCB1、ABCC1、ABCG2和ABCB5基因的表达。

1.11　双荧光素酶报告实验分析miR-9-5p和ONECUT2的靶向关系

根据miRNA生物信息学网站TargetScan（http://www.targetscan.org）预测miR-9-5p可能的靶基因为ONECUT2，分析miRNA与ONECUT2之间的作用序列。设计ONECUT2的3′UTR序列，构建psiCHECK2荧光素酶报告载体。ONECUT2的3′UTR质粒或psiCHECK2空质粒联合miR-9-5p mimic或对照miRNA mimic转染MDA-231细胞，48 h后应用双荧光素酶报告基因检测试剂盒测定细胞荧光素酶活性，计算萤火虫荧光素/海肾荧光素比值。

1.12　Kaplan-Meier Plotter在线分析乳腺癌组织中miR-9-5p表达对患者OS的影响

在Kaplan-Meier Plotter数据分析平台（www.kmplot.com）中检索收集了TCGA数据库中1 062例乳腺癌患者miR-9-5p的表达与OS情况，对临床分期及分级不做限制。miR-9-5p按中位表达值分为高（n=538）和低（n=524）两组，利用Kaplan-Meier中Log-rank模型，计算出相应P值及风险比（hazard ratio，HR）。

1.13　统计学处理

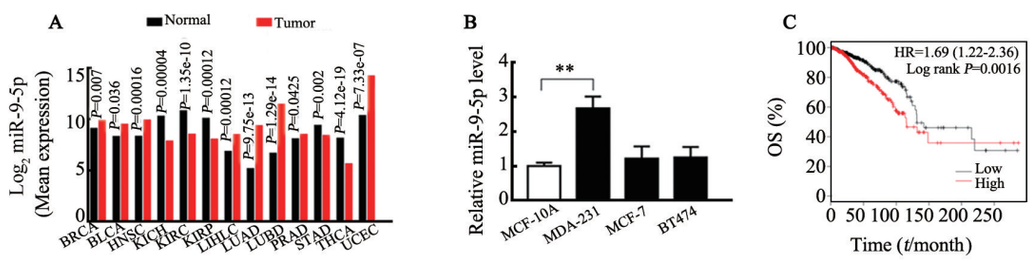
采用GraphPad Prism 7.01软件进行分析，正态分

布的计量资料以$\bar{x}\pm s$表示，两组间比较采用t检验，多组间比较采用One-way ANOVA检验，生存分析绘制生存曲线并进行Log-rank检验，以P<0.05或P<0.01表示差异具有统计学意义。

2 结 果

2.1 miR-9-5p在乳腺癌中呈显著高表达且与患者不良预后有关

应用OncomiR在线数据库检索并分析miR-9-5p在13种不同肿瘤组织与正常组织中的表达差异，结果（图1A）显示，与正常乳腺组织相比，miR-9-5p在乳腺癌组织中呈高表达（P=0.007）。qPCR检测结果（图1B）显示，MDA-231细胞中miR-9-5p表达显著高于正常乳腺细胞MCF-10A（P=0.0005），因此选用MDA-231细胞进行后续研究。Log-rank检验分析结果（图1C）显示，miR-9-5p高表达组患者OS较低表达组患者显著缩短（115.37 *vs* 131.5个月，P=0.0016）。



**P<0.01 *vs* MCF-10A group

A,B: The expression of miR-9-5p in 13 kinds of cancers including BRCA tissues and breast cancer cell lines; C: The prognostic effect of miR-9-5p in 1 062 breast cancer patients. BRCA: Breast invasive carcinoma; BLCA: Bladder urothelial carcinoma; HNSC: Head and neck squamous cell carcinoma; KICH: Kidney chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; PRAD: rostate adenocarcinoma; STAD: Stomach adenocarcinoma; THCA: Thyroid carcinoma; UCEC: Uterine corpus endometrial carcinoma

图1 乳腺癌组织中miR-9-5p的高表达与患者不良预后相关

Fig.1 High expression of miR-9-5p in breast cancer tissues was associated with poor prognosis of patients

2.2 miR-9-5p对乳腺癌干性化的调控

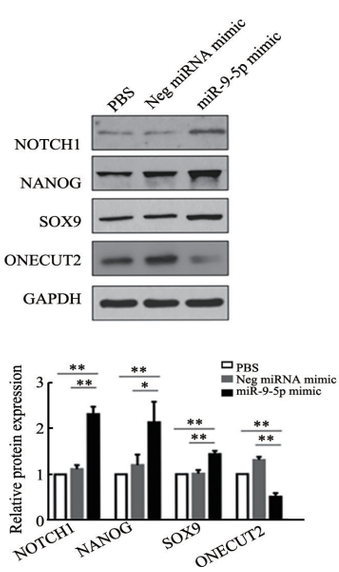
WB检测结果（图2）显示，与阴性、空白对照组相比，miR-9-5p过表达组MDA-231细胞中干性基因NOTCH1、NANOG和SOX9的蛋白表达水平均显著升高（P<0.01或P<0.05），ONECUT2表达显著下调（P<0.01）。

2.3 miR-9-5p与ONECUT2的靶向关系

软件TargetScan的预测结果（图3A）显示，ONECUT2可能是miR-9-5p的靶基因。双荧光素酶实验检测结果（图3B）表明，与转染空白质粒相比，共转染含ONECUT2 3′UTR的质粒及miR-9-5p mimic组的萤火虫荧光素/海肾荧光素比值显著下调（P<0.01），而转染psiCHECK2空白质粒组则无明显变化。

2.4 ONECUT2参与miR-9-5p对肿瘤干性化特征的调控

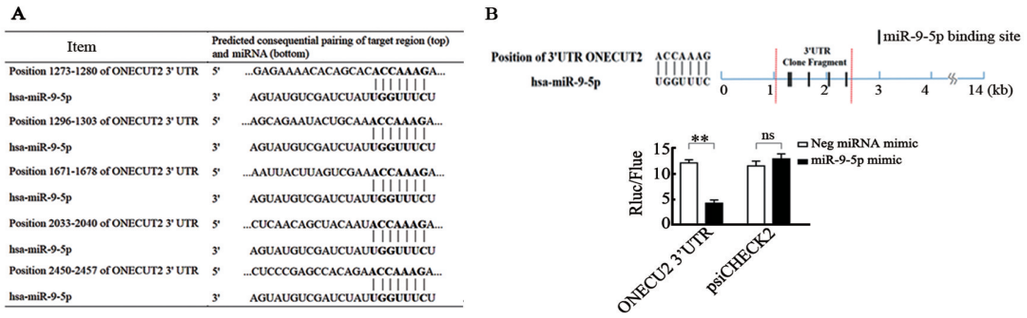
WB检测结果（图4A）显示，转染ONECUT2 siRNA组MDA-231细胞中NOTCH1、NANOG和SOX9蛋白的表达水平较空白、阴性对照组均显著升高（均P<0.01）。FACS（图4B）和WB（图4C）结果显示，miR-9-5p mimic组MDA-231细胞中ALDH$^+$细胞比例较对照组显著升高（P<0.01），NOTCH1、NANOG和SOX9蛋白的表达水平升高（均P<0.01）；在MDA-231/ONECUT2细胞中，miR-9-5p mimic组ALDH$^+$细胞比例与对照组无显著差异（P>0.05），NOTCH1、NANOG和SOX9蛋白表达水平也无显著差异（P>0.05）。



*P<0.05, **P<0.01

图2 miR-9-5p过表达影响MDA-231细胞中干性基因编码蛋白的表达水平

Fig.2 miR-9-5p over-expression regulated the expression level of proteins encoded by stem genes

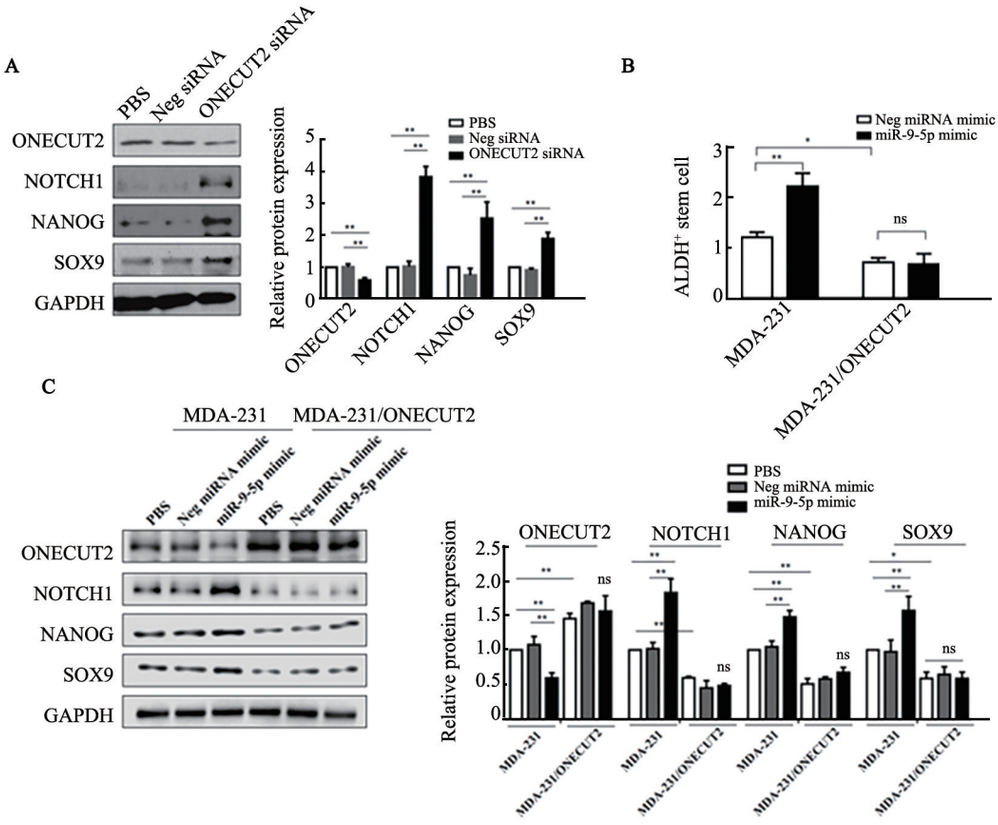


$^{**}P$<0.01 *vs* Neg miRNA mimic group

A: ONECUT2 was predicted to be the putative target gene of miR-9-5p by Targetscan; B: miR-9-5p negatively regulated the expression of ONECUT2 gene

图3 miR-9-5p靶向负调控ONECUT2基因表达

Fig.3 miR-9-5p could target and negatively regulate ONECUT2 gene expression



$^{*}P$<0.05, $^{**}P$<0.01

A: Knock-down of ONECUT2 in MDA-231 promoted the expression of stemness-related genes (NOTCH1, NANOG and SOX9); B: Effect of miR-9-5p or ONECUT2 over-expression on the proportion of ALDH^{+} MDA-231 cells; C: Effect of miR-9-5p or ONECUT2 over-expression on the protein expression of ONECUT2, NOTCH1, NANOG and SOX9 in MDA-231 cells

图4 miR-9-5p/ONECUT2轴参与调控肿瘤细胞MDA-231的干性化特征

Fig.4 miR-9-5p/ONECUT2 axis regulated cancer stem-like properties in MDA-231 cells

2.5　miR-9-5p/ONECUT2轴对MDA-231细胞凋亡、增殖和化疗耐药的影响

FACS检测结果（图5A、B）显示，与相应对照组相比，miR-9-5p过表达组和ONECUT2低表达组MDA-231细胞的凋亡水平均显著下降（均P<0.01），而增殖水平无显著差异（均P>0.05）。

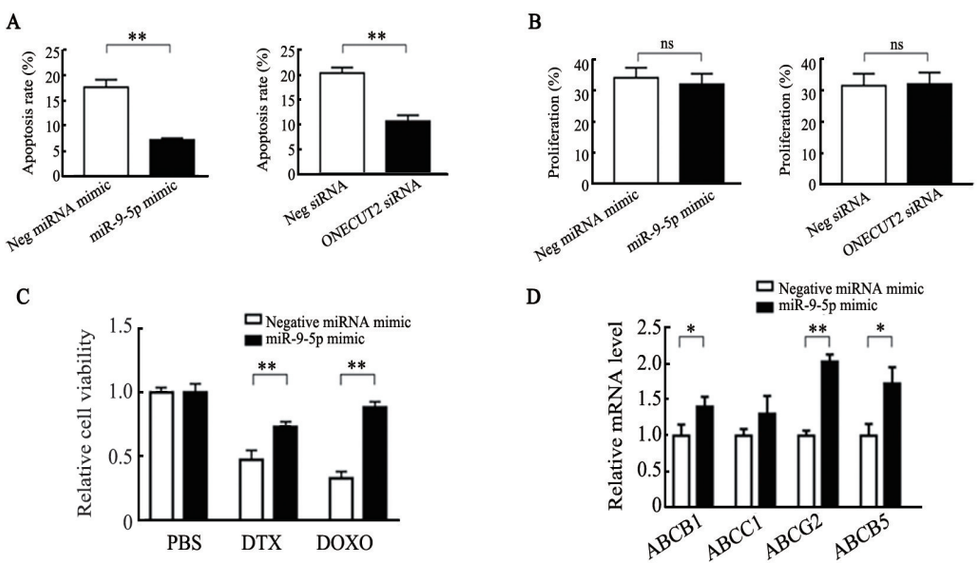
MTS实验检测结果（图5C）显示，在DTX和DOXO干预下，miR-9-5p mimic组MDA-231细胞活力较对照组显著升高（均P<0.01）。

qPCR检测结果（图5D）表明，miR-9-5p mimic组

MDA-231细胞中ABCB1、ABCG2及ABCB5的mRNA水平表达较对照组显著上调（$P<0.05$或$P<0.01$）。

2.6 ONECUT2对NSG小鼠荷瘤模型的影响

WB检测结果（图6A）显示，MDA-231/ONECUT2细胞中ONECUT2蛋白表达水平较MDA-231细胞显著升高（$P<0.01$），说明成功建立了ONECUT2稳定过表达细胞系。分析NSG荷瘤小鼠化疗模型，与接种野生型MDA-231细胞移植瘤的小鼠相比，MDA-231/ONECUT2组小鼠接受DTX治疗后，移植瘤体积较对照组显著降低（图6B，$P<0.05$）；移植瘤组织中NOTCH1、SOX9蛋白表达（NOTCH1：$P=0.0257$；SOX9：$P=0.0185$；图6C）和ABC转运蛋白的mRNA表达显著降低（ABCB1：$P=0.0024$；ABCC1：$P=0.0043$；ABCG2：$P=0.0113$；ABCB5：$P=0.0139$；图6D）。

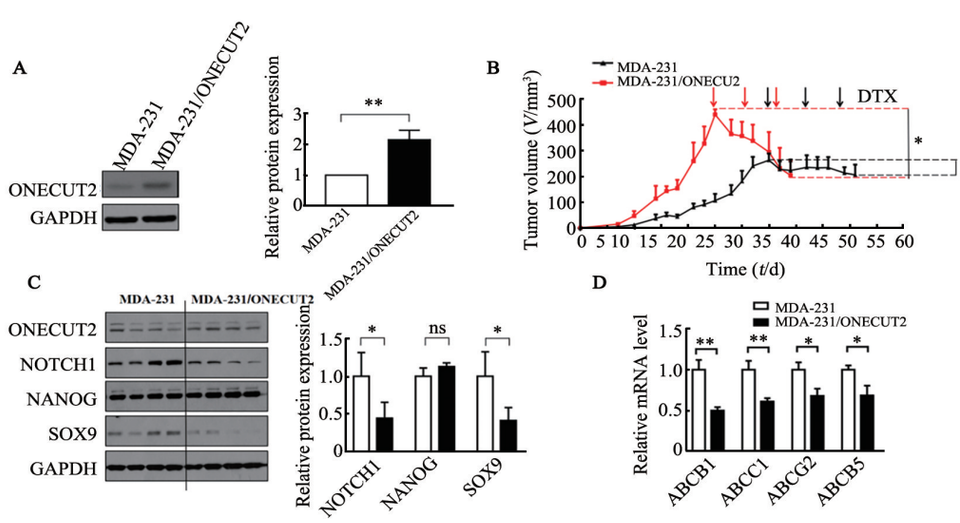


*$P<0.05$, **$P<0.01$ *vs* Neg miRNA mimic or Neg siRNA

A, B: Effects of miR-9-5p over-expression or knock-down of ONECUT2 expression on apoptosis (A) and proliferation (B) of MDA-231 cells; C: Over-expression of miR-9-5p increased the resistance of MDA-231 cells to DTX or DOXO; D: Over-expression of miR-9-5p increased the mRNA levels of ABCB1, ABCG2 or ABCB5 in MDA-231 cells

图5 miR-9-5p/ONECUT2轴对MDA-231细胞凋亡、增殖和化疗耐药的影响

Fig.5 Effects of miR-9-5p/ONECUT2 axis on apoptosis, proliferation and chemo-resistance of MDA-231 cells



*$P<0.05$,**$P<0.01$

A: ONECUT2 stable over-expression cell line MDA-231/ONECUT2 was successfully established; B: ONECUT2 over-expression reduced the resistance of breast cancer tumor-bearing NSG mice to DTX chemotherapy; C: The effect of ONECUT2 over-expression on the protein expressions of NOTCH1, NANOG and SOX9 in breast cancer transplanted tumor tissues of NSG mice; D: The effect of ONECUT2 over-expression on the mRNA expressions of ABCB1, ABCC1, ABCG2 and ABCB5 in breast cancer transplanted tumor tissues of NSG mice

图6 体内实验探讨ONECUT2调控乳腺癌恶性生物学行为

Fig.6 ONECUT2 regulated malignant biological behaviors of breast cancer *in vivo*

3 讨 论

miR-9-5p在肿瘤进程中发挥着重要的调控作用，在多种人类肿瘤中表达各异，在不同肿瘤组织中也发挥着不同的功能。miR-9-5p在乳腺癌[3,7]等肿瘤中呈现显著高表达，可作为促癌miRNA调控肿瘤生物学行为。而在胃癌和卵巢癌等肿瘤中，miR-9-5p则作为抑癌因子发挥抗肿瘤免疫效应[8-10]。事实上，miR-9-5p可通过调控不同的靶基因参与乳腺癌增殖、侵袭和肺癌转移及血管生成过程[5,11]。有研究[6,12-13]表明，miR-9-5p作为影响乳腺癌预后的重要因素，其高表达预示着患者预后不良。在本研究中也发现，miR-9-5p高表达预示乳腺癌患者总生存期缩短。然而，miR-9-5p是否参与调控乳腺癌干性化特征、化疗抵抗、增殖及凋亡功能等恶性生物学行为及具体调控机制有待进一步探索。

本研究以乳腺癌MDA-231细胞系为对象，研究发现，miR-9-5p过表达诱导了肿瘤干细胞化特征，而细胞凋亡率则明显降低。ABC转运蛋白可介导肿瘤化疗耐药[14-15]。本研究发现miR-9-5p过表达可上调ABC转运蛋白表达，增强MDA-231细胞的化疗耐受性。进一步研究发现，ONECUT2作为miR-9-5p的作用靶基因在上述肿瘤生物学行为中发挥着调控作用。NSG小鼠体内实验结果亦表明，过表达ONECUT2抑制了MDA-231细胞干细胞化并降低了细胞的化疗耐药性。

ONECUT2是参与多向潜能分化的转录因子，在发育中的肝脏、神经系统及肠内胚层中均有表达。在肿瘤微环境中，ONECUT2在肿瘤增殖及迁移能力、EMT进程、肿瘤血管生成中发挥重要的调控作用[4,16-18]。在雄激素抵抗性前列腺癌组织中，ONECUT2表达活性增加，干扰雄激素受体蛋白的活性，使前列腺癌的生长并不再依赖于雄激素，增加其对激素疗法的抵抗性[19]。在神经内分泌性前列腺癌中，ONECUT2参与调控缺氧信号通路，促进了肿瘤恶性生物学进程[20]。有研究[21-24]显示，促瘤性miRNA，包括miR-9、miR-429和miR-218，均可靶向调控ONECUT2，在肿瘤微环境中发挥关键调控作用。此外，miR-9在脂肪代谢[25]以及促脑部血管生成[26]等过程中亦直接参与调控ONECUT2表达。尽管如此，ONECUT2在乳腺癌微环境中所起的作用仍有待进一步明确。ZHANG等[27]研究表明，乳腺癌患者血清中高表达5种miRNA（miR-30b-5p、miR-96-5p、miR-182-5p、miR-374b-5p和miR-942-5p），它们均能够靶向ONECUT2，低表达的ONECUT2与患者不良预后呈现正相关。本研究发现，ONECUT2低表达诱导肿瘤干性化及化疗耐药性，减低细胞凋亡能力，为研究乳腺癌发生发展提供了新的思路。

综上所述，本研究通过体外和体内实验发现，乳腺癌中高表达的miR-9-5p通过靶向下调ONECUT2表达，促进肿瘤干细胞化进程，诱导了其对化疗药物的耐受性及抗凋亡能力。miR-9-5p及其靶基因ONECUT2有望为乳腺癌的临床治疗提供新的思路。然而，乳腺癌中miR-9-5p可能存在其他的作用靶基因参与肿瘤干细胞化等恶性生物学行为的调控，同时ONECUT2调控乳腺癌生物学行为的具体机制未能彻底阐明，这些问题还有待进一步深入探索。

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［收稿日期］ 2020-09-06 ［修回日期］2020-10-18
［本文编辑］ 黄静怡