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在线全文

·论 著·

circ_0084043/miR-31-5p调节HMGA1参与动脉粥样硬化作用的机制研究*

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【摘要】 目的 探究circ_0084043/miR-31-5p调节HMGA1参与动脉粥样硬化（atherosclerosis, AS）作用的具体机制。**方法** 收集2020年9月–2023年9月49例AS患者的颈动脉斑块及血管组织与对应斑块旁正常组织，qRT-PCR检测circ_0084043、miR-31-5p和HMGA1表达。通过CCK-8、克隆形成、划痕、Transwell实验检测氧化型低密度脂蛋白（oxidized low-density lipoprotein, ox-LDL）诱导人血管平滑肌细胞（vascular smooth muscle cells, VSMCs）增殖、迁移、侵袭能力，通过生物信息学、双荧光素酶报告基因检测验证circ_0084043、miR-31-5p、HMGA1之间的靶向调节关系。建立AS小鼠模型，通过油红O、HE染色观察主动脉病变情况，生化检测静脉血血脂指标，qRT-PCR检测miR-31-5p、HMGA1表达情况，免疫组化检测主动脉HMGA1、α-SMA表达情况。**结果** AS患者斑块、病变血管组织circ_0084043、HMGA1表达水平升高（$P<0.05$），miR-31-5p表达水平下降（$P<0.05$）。circ_0084043与miR-31-5p表达水平呈负相关（$r=-0.3855$，$P=0.0062$），HMGA1与circ_0084043表达水平呈正相关（$r=0.3317$，$P=0.0199$），与miR-31-5p表达水平呈负相关（$r=-0.3351$，$P=0.0186$）。miR-31-5p与circ_0084043、HMGA1存在靶向结合位点。与对照组相比，sh-NC组、scramble组、circ_0084043+miR-31-5p mimics组、miR-31-5p inhibitor+sh-*HMGA1*组、circ_0084043+sh-*HMGA1*组增殖、克隆形成、迁移、侵袭能力升高（$P<0.05$）；与sh-NC组相比，sh-0084043组则降低（$P<0.05$）；miR-31-5p mimics组、sh-*HMGA1*组较scramble组增殖、克隆形成、迁移、侵袭能力下降（$P<0.05$）。与sh-NC组相比，sh-0084043组主动脉脂质斑块、坏死核心面积减小（$P<0.05$），TC、TG、LDL-C、HMGA1 mRNA及蛋白、α-SMA表达水平下降（$P<0.05$），HDL-C、miR-31-5p表达水平上升（$P<0.05$）。**结论** circ_0084043通过抑制miR-31-5p提高HMGA1表达促进VSMCs增殖、迁移，推动AS进展。

【关键词】 circ_0084043 miR-31-5p 高迁移率族蛋白A1 动脉粥样硬化 血管平滑肌细胞

circ_0084043/miR-31-5p Is Involved in Atherosclerosis by Regulating HMGA1: Investigation of the Mechanisms

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【Abstract】 Objective To explore the specific mechanism by which circ_0084043/miR-31-5p regulates high mobility group AT-hook 1 (HMGA1) and thus participates in atherosclerosis (AS). **Methods** Carotid plaque tissues, diseased vascular tissue, and corresponding normal tissue from areas adjacent to the plaques were collected from 49 AS patients between September 2020 and September 2023. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to determine circ_0084043 expression. The proliferation, migration, and invasion of vascular smooth muscle cells (VSMCs) induced by oxidized low-density lipoprotein (ox-LDL) were determined by CCK-8, clone formation, scratch, and Transwell assays. The targeted regulatory relationship among circ_0084043, miR-31-5p, and HMGA1 was verified by bioinformatics and dual luciferase reporter gene assay. An AS mouse model was established, and the pathological lesions in the aorta of AS model mice were observed by oil red O and HE stainings. Biochemical testing was performed to assess blood lipids in venous blood. qRT-PCR was used to determine the expression of miR-31-5p and HMGA1. Immunohistochemistry was performed to assess the expression of HMGA1 and α-smooth muscle actin (α-SMA) in the aorta of the AS model mice. **Results** In AS patients, the expression levels of circ_0084043 and HMGA1 in carotid plaque tissues and diseased vascular tissues were elevated ($P<0.05$), while the expression level of miR-31-5p was decreased ($P<0.05$). The expression of circ_0084043 was negatively correlated with the expression of miR-31-5p ($r=-0.3855$, $P=0.0062$). HMGA1 expression was positively correlated with circ_0084043 expression ($r=0.3317$, $P=0.0199$), and negatively correlated with miR-31-5p ($r=-0.3351$, $P=0.0186$). MiR-31-5p had target binding sites for both circ_0084043 and HMGA1. Compared with the control group, the proliferation, clone formation, migration, and invasion abilities of the sh-NC group, the scramble group, the circ_0084043+miR-31-5p mimics group, the miR-31-5p inhibitor+sh-*HMGA1* group, and the circ_0084043+sh-*HMGA1* group increased ($P<0.05$). In contrast, the proliferation, clone formation,

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migration, and invasion abilities of the sh-0084043 group significant decreased compared to those of the sh-NC group (P<0.05). The proliferation, clone formation, migration, and invasion abilities of the miR-31-5p mimics group and the sh-*HMGA1* group decreased compared with those of the scramble group (P<0.05). Compared with the sh-NC group, sh-0084043 group had decreased aortic lipid plaque and necrotic core areas (P<0.05). Total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and the expression levels of HMGA1 mRNA and protein and α-SMA decreased (P<0.05), while high-density lipoprotein cholesterol (HDL-C) and miR-31-5p expression levels increased (P<0.05). **Conclusion** circ_0084043 promotes the proliferation and migration of VSMCs and promotes the progress of AS by inhibiting miR-31-5p and increasing the expression of HMGA1.

【Key words】 circ_0084043 miR-31-5p High mobility group AT-hook 1 Atherosclerosis Vascular smooth muscle cell

动脉粥样硬化(atherosclerosis, AS)是以动脉壁形成纤维脂肪病变为特征的慢性炎症性疾病,是引发冠状动脉疾病、心肌梗死等疾病的病理基础[1-2]。尽管目前在AS的治疗方面已取得了重大进展,但AS性心血管疾病在全球人口死亡原因中仍占据显著地位[3]。血管主要由血管平滑肌细胞(vascular smooth muscle cells, VSMCs)、内皮细胞和成纤维细胞等组成,VSMCs主要位于血管壁的中层,围绕着内皮细胞形成环状排列,具有收缩和舒张的能力,通过控制血管直径和血管壁的张力来调节血压和血流量[4-5]。血管炎症、内皮功能障碍和VSMCs异常增殖/迁移为AS病变发展的重要驱动因素[6],因此,探究VSMCs参与AS病变的详细机制对AS的防治具有重要的意义。circRNA与线性RNA相较更为稳定和保守,具有调节选择性剪接、亲本基因表达、海绵样吸附miRNA等生物学功能[7-8],相关研究显示,已有多种circRNAs参与心肌修复、肺动脉高压、AS等多种疾病进展[9-11]。circ_0084043是一种新发现的circRNA,在黑色素瘤细胞中表达上调[12],但其在AS病变进展中的作用尚不清楚。miR-31-5p与circ_0084043存在结合位点,参与调节肾细胞癌、非小细胞肺癌的迁移过程[13-15],但在VSMCs迁移中的作用有待研究。HMGA1是HMG家族的成员之一,参与调节多种炎症反应相关基因,与AS斑块形成相关[16]。本次研究通过体内外实验观察circ_0084043/miR-31-5p调节HMGA1参与AS的具体作用机制,现报告如下。

1 材料与方法

1.1 组织样本

收集2020年9月–2023年9月共49例鉴定为Ⅳ ~ Ⅴ期病变[17]的AS患者颈动脉斑块、病变血管组织与对应斑块旁正常组织。患者男性31例,女性18例,年龄59 ~ 78(69.63±6.12)岁。本研究经过宁夏医科大学总医院医学伦理委员会批准(批准号2020-660)。

1.2 实验动物

12只雄性6周龄SPF级C57BL/6J ApoE$^{-/-}$小鼠,体质量(21±2) g,购自宁夏医科大学实验动物中心。实验操作符合3R原则。

1.3 实验细胞

人VSMCs(货号BW-5067)、人胚肾细胞HEK 293T(货号BTCC-1003),购自北京博沃尔斯生物科技有限公司。

1.4 主要试剂

TRIzol试剂(美国Thermo Fisher,美国),核糖核酸酶R(ribonuclease R, RNase R,瑞必泰,中国),PCR引物(晶莱生物,中国),慢病毒表达载体(珠海舒桐医疗科技,中国),MTT检测试剂盒(囊萤科技,中国),油红O染色试剂盒、HE染色试剂盒(碧云天,中国),总胆固醇(total cholesterol, TC)、甘油三酯(triglyceride, TG)、低密度脂蛋白胆固醇(low-density lipoprotein cholesterol, LDL-C)、高密度脂蛋白胆固醇(high-density lipoprotein cholesterol, HDL-C)(南京建成生物工程研究所,中国),HMGA1抗体、α平滑肌肌动蛋白(α-smooth muscle actin, α-SMA)抗体(Abcam,英国)。

1.5 qRT-PCR检测

提取AS患者病变及正常组织、VSMCs总RNA,将组织与细胞的总RNA反转录成cDNA,配制PCR反应体系。反应条件:95 ℃预变性2 min,然后进行40个循环的扩增,每个循环包括95 ℃变性15 s,60 ℃退火、延伸60 s,引物序列见表1。应用$2^{-\Delta\Delta Ct}$法计算基因的表达水平。

表 1 引物序列

Table 1 Primer sequence

Primer	Sequence (5'-3')
circ_0084043	F: TTCTAGACAGCCGGGGAGTG
	R: CCAAAACCTTTCTTTCTTGATGGGA
miR-31-5p	F: GCCGCAGGCAAGATGCTGGC
	R: CAGTGCAGGGTCCGAGGT
HMGA1	F: AAGGGGCAGACCCAAAAA
	R: TCCAGTCCCAGAAGGAAGC
GAPDH	F: ACCACAGTCCATGCCATCAC
	R: TCCACCACCCTGTTGCTGTA
U6	F: GCGCGTCGTGAAGCGTTC
	R: GTGCAGGGTCCGAGGT

1.6 细胞培养

使用含10%胎牛血清、1%双抗的McCoy's 5A培养基，于37 ℃、5%CO_2培养箱中培养VSMCs。除正常培养的对照组外，其余分组培养基中均添加100 μg/mL氧化型低密度脂蛋白（oxidized low-density lipoprotein, ox-LDL）以模拟AS环境。VSMCs分为对照组、sh-NC组、sh-0084043组、scramble组（转染sh-NC+miR-NC）、miR-31-5p mimics组、circ_0084043+miR-31-5p mimics组（转染circ_0084043+miR-31-5p mimics）、sh-*HMGA1*组、miR-31-5p inhibitor+sh-*HMGA1*组（转染miR-31-5p inhibitor+sh-*HMGA1*）、circ_0084043+sh-*HMGA1*组（转染circ_0084043+sh-*HMGA1*），按照分组进行培养与慢病毒转染，72 h后收集细胞用于后续实验。

1.7 CCK-8检测

通过CCK-8检测不同剂量ox-LDL对VSMCs的细胞毒性作用。将VSMCs分为0、25、50、75、100、125、150 μg/mL组，按照分组名称向培养基中加入相应剂量的ox-LDL，正常培养24、48、72 h后，加入10 μL CCK-8试剂，避光培养2 h，酶标仪450 nm测定吸光度。

1.8 克隆形成实验

6孔板接种各组细胞，培养10 d。轻轻弃去孔板中的培养基，每孔加入适量预冷甲醇，确保细胞层被完全覆盖，固定30 min。弃去甲醇，用去离子水或PBS轻轻洗涤细胞层，每孔加入1%结晶紫染色液，室温染色20 min。去除多余的染色液，待孔板干燥后统计每个孔中的克隆数量。

1.9 划痕实验

6孔板接种各组细胞，观察细胞覆盖率达到约90%，使用无菌移液器枪头在每个孔的细胞层上制造划痕。将孔板置于培养箱中继续培养24 h，于划痕后（定义为0 h）以及培养24 h后测量划痕宽度，计算迁移率。

1.10 Transwell实验

调整各组细胞制成悬液，滴加至底部涂抹了基质胶的小室上室中，下室加入含血清的DMEM培养基，将小室放回培养箱中，在相同的培养条件下培养24 h。去除未穿透上室膜的细胞，加入体积分数4%多聚甲醛，对小室底部的细胞进行固定，固定时间30 min。用PBS清洗小室，并用结晶紫对细胞进行染色。将染色后的小室置于光学显微镜下观察，记录穿过滤膜的细胞数量。

1.11 双荧光素酶报告基因检测

通过生物信息学分析预测获得了miR-31-5p分别与circ_0084043、HMGA1的结合位点。为了验证预测结果，分别设计了psiCheck2-0084043-Luc、psiCheck2-0084043-MUT-Luc、psiCheck2-HMGA1-Luc、psiCheck2-HMGA1-MUT-Luc四种荧光质粒，将这些质粒与miR-31-5p模拟物共同转染到HEK 293T细胞中，通过检测荧光素酶活性来评估它们之间的相互作用。

1.12 建立AS模型

将小鼠随机分成sh-NC组与sh-0084043组两个实验组，每组各6只。两组小鼠均采用一种高脂饲料进行喂养，该饲料含有40 kal（1 kal=4.184 kJ）脂肪、1.25%胆固醇和0.5%胆酸，喂养周期为16周[18]。喂养至第11周，通过尾静脉注射的方式进行基因干预：sh-NC组尾静脉注射sh-NC慢病毒，sh-0084043组尾静脉注射sh-0084043慢病毒，注射频率为1次/周。处死所有小鼠，收集静脉血和主动脉组织样本进行后续分析。

1.13 主动脉组织病理学

截取部分主动脉组织，体积分数4%多聚甲醛固定后制成石蜡切片。依据油红O和HE染色试剂盒说明书的操作步骤对组织、石蜡切片进行染色，于光镜下观察拍照。

1.14 小鼠血脂指标检测

分离静脉血液样本的血清，根据生化检测试剂盒的指导对血清样本进行必要的预处理，随后严格按照试剂盒的操作说明进行实验，确保每一步骤的准确性。检测血清TC、TG、LDL-C、HDL-C水平。

1.15 小鼠主动脉免疫组化检测

取主动脉石蜡切片，去除石蜡、复水、暴露抗原表位、封闭，分别加入1∶100稀释的HMGA1、α-SMA抗体，4 ℃孵育过夜令目标抗原充分结合。次日加入生物素标记的二抗，在室温下孵育30 min。加入链霉亲和素酶进一步放大信号，室温孵育30 min，经过显色反应后，复染切片增加对比度，随后进行脱水、透明处理并封片，于光镜下拍照。

1.16 统计学方法

采用SPSS 24.0统计软件作为分析工具，计量资料以$\bar{x}\pm s$表示，两组间比较采用独立样本t检验，多组间比较采用单因素方差分析。α=0.05。

2 结果

2.1 沉默circ_0084043对ox-LDL诱导VSMCs增殖、迁移与侵袭能力的影响

结果见图1。qRT-PCR检测结果显示，AS组织circ_0084043表达水平高于正常组织（P<0.05）。不同剂量ox-LDL处理后，100 μg/mL ox-LDL处理72 h后的VSMCs细胞增殖活力最高（P<0.05），因此后续细胞实验选择该质量浓度ox-LDL诱导VSMCs；与0 μg/mL ox-LDL组相比，

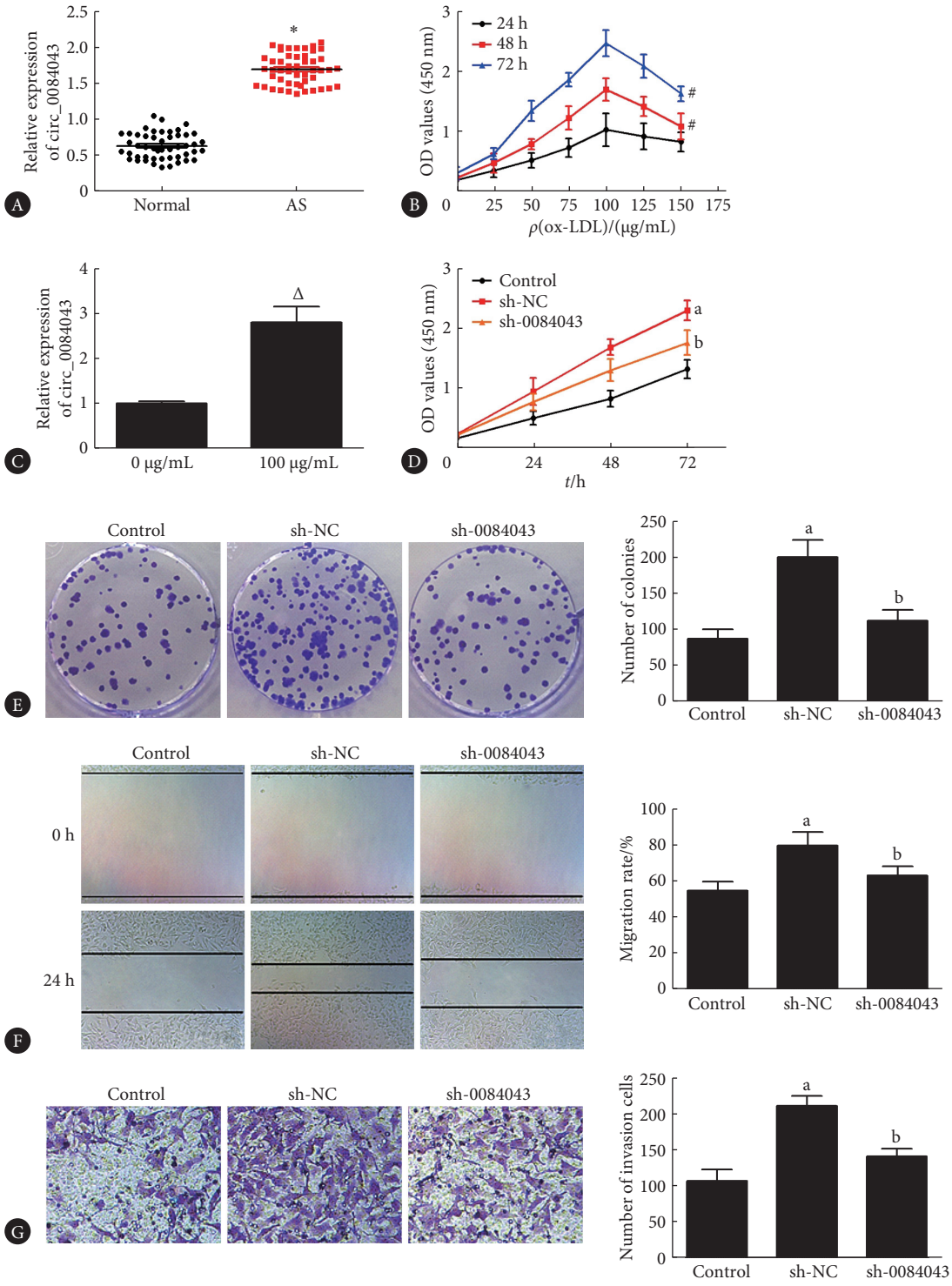

图 1 circ_0084043在AS中的表达以及沉默circ_0084043对ox-LDL诱导VSMCs增殖、迁移与侵袭能力的影响

Fig 1 Expression of circ_0084043 in AS and the effect of silencing circ_0084043 on the proliferation, migration, and invasion of VSMCs induced by ox-LDL

A, qRT-PCR detection of circ_0084043 in AS patients; B, cell toxicity assay results; C, qRT-PCR detection of circ_0084043 in VSMCs; D, MTT assay results; E, clone formation assay results; F, scratch assay results; G, Transwell assay results (orginal magnification ×100). * P<0.05, vs. normal; # P<0.05, vs. 24 h; Δ P<0.05, vs. 0 μg/mL. a P<0.05, vs. control; b P<0.05, vs. sh-NC. n=3.

100 μg/mL ox-LDL组circ_0084043表达水平升高(P<0.05)。相较于对照组，sh-NC组在增殖、克隆形成、迁移以及侵袭等能力上均有所增强(P<0.05)；进一步与sh-NC组进行对比，sh-0084043组则表现出明显的降低趋势(P<0.05)。

2.2 circ_0084043靶向miR-31-5p对ox-LDL诱导VSMCs增殖、迁移与侵袭能力的影响

结果见图2。qRT-PCR检测结果显示，AS病变组织

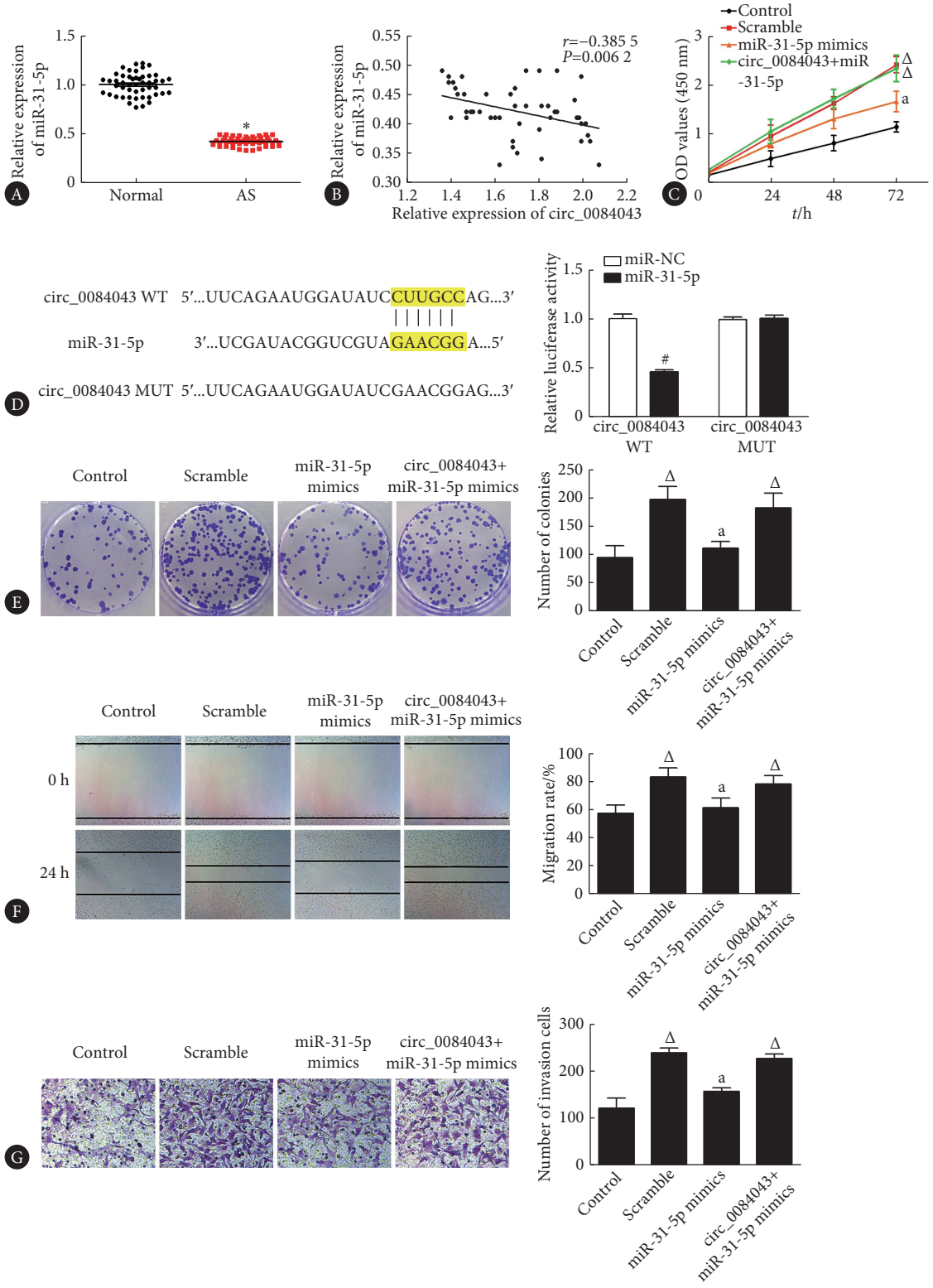

图 2　circ_0084043靶向miR-31-5p对ox-LDL诱导VSMCs增殖、迁移与侵袭能力的影响

Fig 2　The effect of circ_0084043 targeting miR-31-5p on the proliferation, migration and invasion of VSMCs treated with ox-LDL

A, qRT-PCR detection of miR-31-5p in AS patients; B, results of correlation analysis; C, MTT assay results; D, results of the dual-luciferase reporter gene assay; E, clone formation assay results; F, scratch assay results; G, Transwell assay results (orginal magnification ×100). * P<0.05, vs. normal; # P<0.05, vs. miR-NC; Δ P<0.05, vs. control; a P<0.05, vs. scramble. n=3.

miR-31-5p表达水平较正常组织降低(P<0.05)。circ_0084043与miR-31-5p表达呈负相关(r=−0.385 5, P=0.006 2)。circ_0084043与miR-31-5p存在靶向结合位点，其野生型荧光信号强度被miR-31-5p降低(P<0.05)。与对照组相

比, scramble组、circ_0084043+miR-31-5p mimics组增殖、克隆形成、迁移和侵袭能力均增强(P<0.05); miR-31-5p mimics组较scramble组均下降(P<0.05)。

2.3 circ_0084043/miR-31-5p调节HMGA1对ox-LDL诱导VSMCs增殖、迁移与侵袭能力的影响

结果见图3。qRT-PCR检测结果显示, AS病变组织

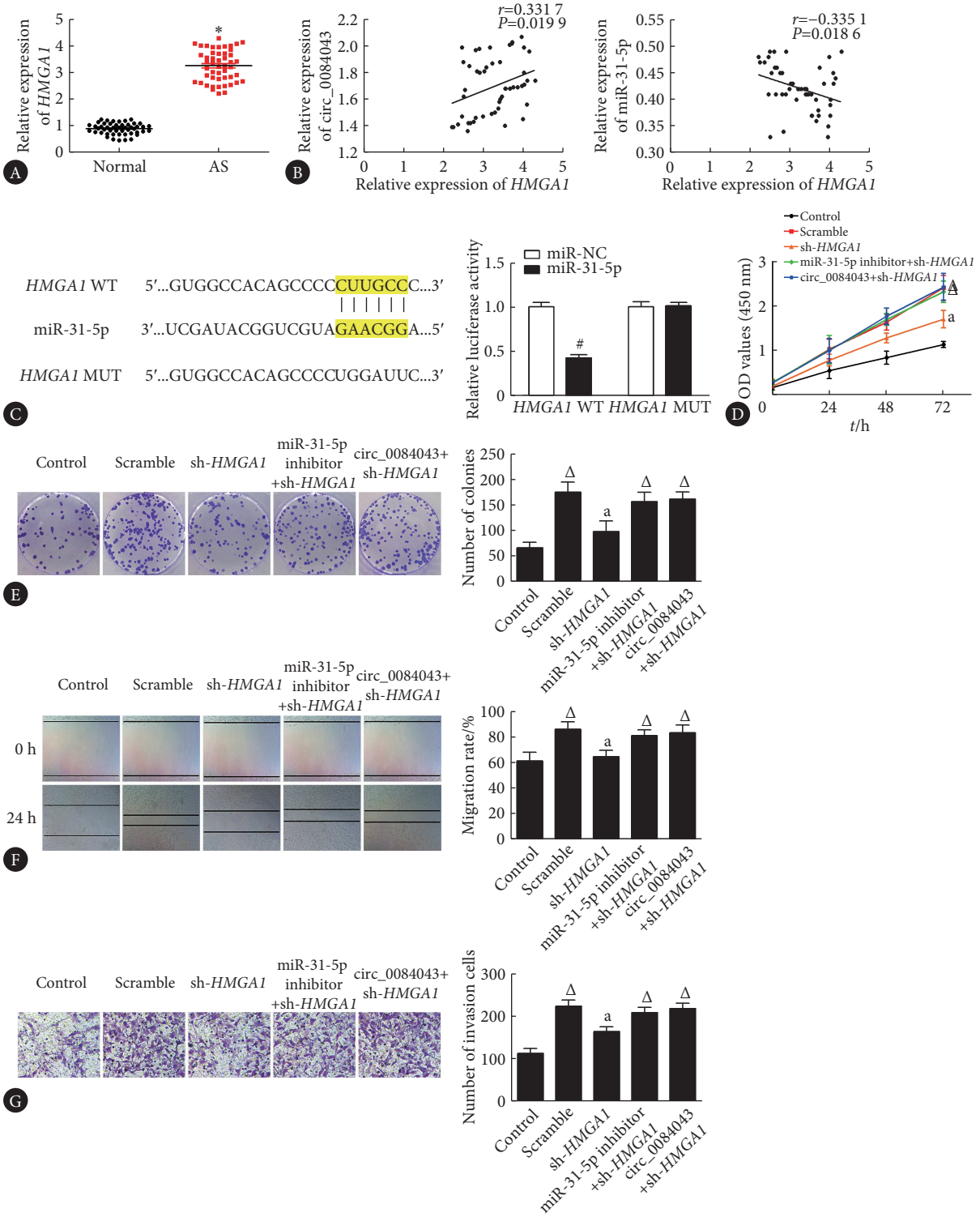

图 3 circ_0084043/miR-31-5p调节HMGA1对ox-LDL诱导VSMCs增殖、迁移与侵袭能力的影响

Fig 3 Effects of circ_0084043/miR-31-5p regulation of HMGA1 on the proliferation, migration and invasion of VSMCs treated with ox-LDL

A, qRT-PCR detection of *HMGA1* in AS patients; B, results of correlation analysis; C, results of the dual-luciferase reporter gene assay; D, MTT assay results; E, clone formation assay results; F, scratch assay results; G, Transwell assay results (orginal magnification ×100). * P<0.05, vs. normal; # P<0.05, vs. miR-NC; Δ P<0.05, vs. control; a P<0.05, vs. scramble. n=3.

*HMGA1*表达水平较正常组织升高(P<0.05)。相关性分析结果显示, HMGA1与circ_0084043表达水平呈正相关(r=0.331 7, P=0.019 9), 与miR-31-5p表达水平呈负相关(r=−0.335 1, P=0.018 6), miR-31-5p靶向HMGA1(P<0.05)。与对照组相比, scramble组、miR-31-5p inhibitor+sh-*HMGA1*组、circ_0084043+sh-*HMGA1*组增殖、克隆形成、迁移及侵袭能力均有所上升(P<0.05); 与scramble组相比, sh-*HMGA1*组则下降(P<0.05)。

2.4　沉默circ_0084043抑制小鼠AS进展

结果见图4。与sh-NC组相比, sh-0084043组主动脉脂质斑块、坏死核心面积减小(P<0.05), TC、TG、LDL-C水平下降(P<0.05), HDL-C水平上升(P<0.05), miR-31-

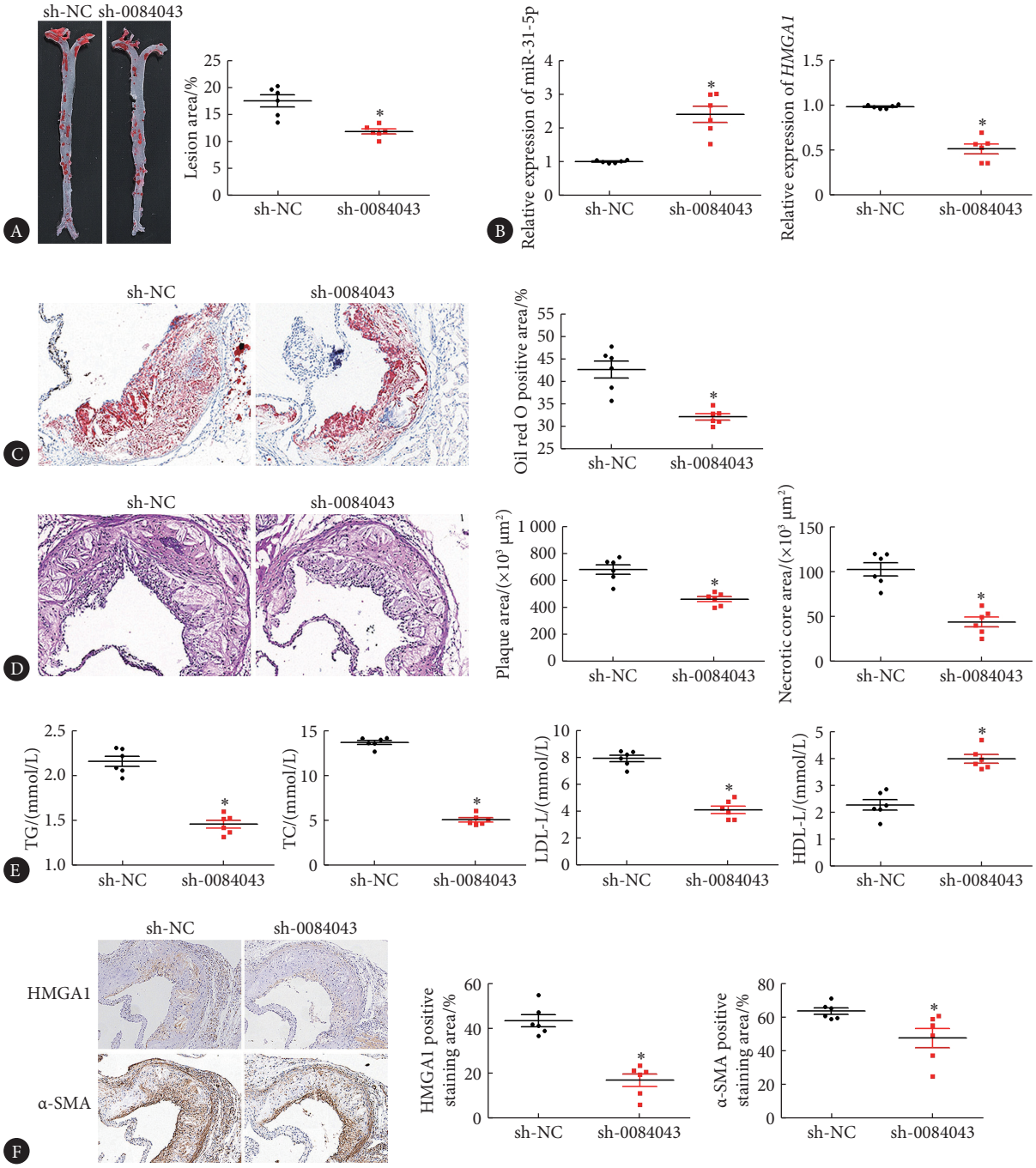

图 4　沉默circ_0084043抑制小鼠AS进展

Fig 4　Silencing circ_0084043 inhibits the progression of AS in mice

A, Oil red O staining of aortic tissues; B, results of qRT-PCR assay; C, oil red O staining on paraffin sections of aorta (orginal magnification ×200); D, HE staining on paraffin sections of aorta (orginal magnification ×200); E, results of blood lipid test (HE staining, orginal magnification ×200); F, immunohistochemical examination of aortic paraffin sections, ×200. * P<0.05, vs. sh-NC. n=6.

5p表达水平升高(P<0.05)，*HMGA1* mRNA、HMGA1蛋白、α-SMA表达水平下降(P<0.05)。

2.5 circ_0084043/miR-31-5p调节HMGA1参与AS的具体作用机制

综合本次研究结果发现，circ_0084043在AS患者颈动脉斑块、病变血管组织、AS小鼠主动脉组织中过表达，抑制circ_0084043表达能够降低VSMCs的增殖、迁移、侵袭能力，推测circ_0084043可能通过靶向miR-31-5p提高HMGA1表达促进AS进展，其可能的作用机制见图5。

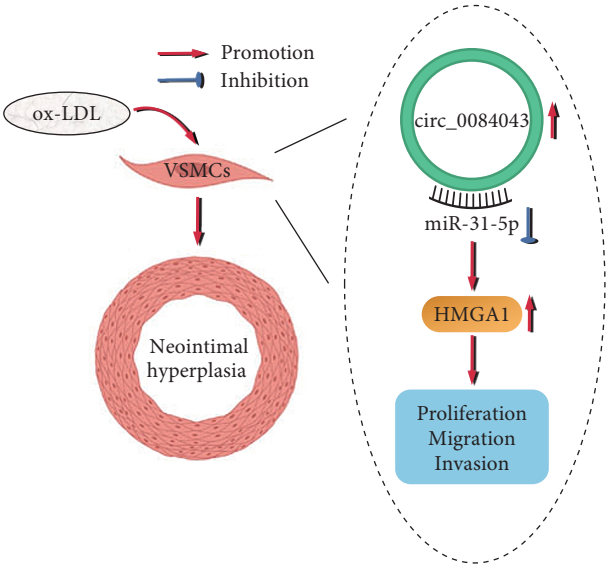

图 5 circ_0084043/miR-31-5p调节HMGA1参与AS的具体作用机制

Fig 5 The specific mechanism by which circ_0084043/miR-31-5p regulates HMGA1 and participates in AS

3 讨论

VSMCs是血管壁中的主要细胞类型，负责维持血管壁的完整性、弹性和收缩性[19]。在健康血管中，VSMCs保持静止、可收缩的表型，但在出现损伤或衰老的情况下，VSMCs收缩标志物表达水平下降，去分化为过度增殖和迁移的合成表型，并转分化为炎性巨噬细胞和钙化的成软骨细胞[20-21]。动脉内皮损伤为AS的始动因素，VSMCs的异常迁移、增殖可致AS斑块形成[22]。近期一项对AS小鼠体内VSMCs谱系追踪的研究表明，AS斑块中30%～70%的细胞群最初来源于VSMCs[23]，因此，揭示VSMCs迁移、增殖的机制对AS的防治有着重要的理论意义。circRNA在基因调控、细胞增殖等过程中发挥关键作用，已有多项研究证明，AS患者血清中不同circRNA的表达水平存在差异[24-25]，HOU等[26]的研究显示，circ_0008896在体外和体内AS模型中均显著上调，抑制circ_0008896表达降低了VSMCs的增殖、迁移和侵袭能力。YANG等[27]的研究表明，沉默circ脂肪酶成熟因子1可通过miR-125a-3p/血管内皮生长因子A或成纤维细胞生长因子1轴抑制血小板源性生长因子-BB诱导人主动脉VSMCs的细胞活力、细胞周期进展和迁移能力。本研究发现，circ_0084043在AS患者斑块、病变组织中表达水平明显升高，沉默circ_0084043后，ox-LDL对VSMCs的影响减弱，AS模型主动脉病变减轻，血脂指标有所改善，提示circ_0084043可能参与VSMCs表型转化，促进斑块形成，可能是AS的潜在生物标志物。生物信息学分析预测与双荧光素酶报告基因检测结果证实circ_0084043能够与预测靶点miR-31-5p相互作用。miR-31-5p在AS患者斑块、病变组织中表达下降，与circ_0084043表达负相关，过表达miR-31-5p抑制了ox-LDL对VSMCs的促进效果，而过表达miR-31-5p的同时过表达circ_0084043逆转了miR-31-5p对AS细胞、动物模型的抑制作用，提示miR-31-5p能够抑制AS发展。

HMGA1是一种染色质非组蛋白，快速生长的细胞中高表达，可诱导染色质聚集，通过染色质重塑调节基因表达模式，参与诱导型转录过程[28]。HMGA1与多种转录元件和染色质结构调节因子发生物理相互作用，协调其在基因启动子和增强子区域的组装，在基因特异性转录调节过程中发挥重要作用[29]。在本研究中，HMGA1在AS患者斑块、病变组织中表达升高，与circ_0084043表达正相关，而与miR-31-5p存在靶向负调控关系。抑制HMGA1表达降低了ox-LDL诱导VSMCs的增殖、迁移与侵袭能力，而抑制miR-31-5p表达或过表达circ_0084043的同时抑制HMGA1表达恢复了AS细胞模型的增殖、迁移、侵袭能力；sh-0084043组主动脉HMGA1、VSMCs标志物α-SMA表达水平下降，提示HMGA1表达受circ_0084043/miR-31-5p调节，对AS发展具有促进作用。

综上所述，circ_0084043在AS患者斑块、病变组织、ox-LDL诱导VSMCs、AS小鼠主动脉中过表达，可能通过抑制miR-31-5p提高HMGA1表达促进VSMCs增殖、迁移，推动AS进展，具有成为AS新的治疗靶点的价值。

* * *

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