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·基础研究·

长链非编码RNA DUXAP9促进头颈鳞癌细胞增殖和转移

周文凯[1,2,3,4,5]，王佳璇[2]，王媛凤[2]，陈萌[6]，陶星如[2]，刘喆麒[1,2,3,4,5]，张煦[1,2,3,4,5]，季彤[1,2,3,4,5]，曹巍[1,2,3,4,5]

1. 上海交通大学医学院附属第九人民医院口腔颌面头颈肿瘤科，上海(200011)；2. 上海交通大学口腔医学院，上海(200011)；3. 国家口腔医学中心，上海(200011)；4. 国家口腔疾病临床医学研究中心，上海(200011)；5. 上海市口腔医学重点实验室，上海(200011)；6. 上海交通大学公共卫生学院，上海(200025)

【摘要】 目的 探讨长链非编码RNA双同源盒假基因9(double homeobox A pseudogene 9，DUXAP9)在头颈鳞癌中的作用，研究DUXAP9在头颈鳞癌细胞中的表达水平、分子功能和机制。**方法** lnc RNA芯片筛选头颈鳞癌组织与癌旁组织差异表达的lncRNA，TCGA数据库中分析DUXAP9在头颈鳞癌组织中的表达水平及其与患者预后的关系；qRT-PCR检测DUXAP9在头颈鳞癌组织样本和细胞系中的表达水平。沉默DUXAP9后，CCK-8实验、细胞划痕实验、Transwell迁移实验和裸鼠皮下成瘤实验评估其在头颈鳞癌细胞中的功能。qRT-PCR和Western blot检测沉默DUXAP9后，头颈鳞癌细胞中上皮-间充质转化(epithelial-mesenchymal transition，EMT)相关蛋白转录及翻译水平的变化。**结果** lncRNA芯片结果显示，与癌旁组织相比，DUXAP9在头颈鳞癌中异常高表达。TCGA数据库分析表明，与DUXAP9低表达患者相比，DUXAP9高表达的患者生存率差；qRT-PCR实验表明DUXAP9在头颈鳞癌组织标本和细胞系中异常高表达。沉默DUXAP9可以显著抑制头颈鳞癌细胞的增殖能力、迁移能力和EMT相关基因的表达水平。沉默DUXAP9能显著抑制头颈鳞癌细胞系CAL27的裸鼠皮下成瘤能力。**结论** DUXAP9促进头颈鳞癌细胞的增殖、迁移和裸鼠皮下成瘤能力。DUXAP9可能通过调控EMT介导头颈鳞癌细胞的迁移能力。

【关键词】 长链非编码RNA；DUXAP9；基因沉默；头颈鳞癌；上皮间质转化；E-钙黏附蛋白；N-钙黏附蛋白；波形蛋白；细胞增殖；细胞迁移；裸鼠皮下成瘤

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Long non-coding RNA DUXAP9 promotes the proliferation and metastasis of head and neck squamous cell carcinoma

ZHOU Wenkai[1,2,3,4,5], WANG Jiaxuan[2], WANG Yuanfeng[2], CHEN Meng[6], TAO Xingru[2], LIU Zheqi[1,2,3,4,5], ZHANG Xu[1,2,3,4,5], JI Tong[1,2,3,4,5], CAO Wei[1,2,3,4,5]. 1. Department of Oral and Maxillofacial & Head and Neck Oncology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200011, China; 2. College of Stomatology, Shanghai Jiao Tong University, Shanghai 200011, China; 3. National Center for Stomatology, Shanghai 200011, China; 4. National Clinical Research Center for Oral Diseases, Shanghai 200011, China; 5. Shanghai Key Laboratory of Stomatology, Shanghai 200011, China; 6. College of Public Health, Shanghai Jiao Tong University School, Shanghai 200025, China

Corresponding author: CAO Wei, Email: caowei561521@hotmail.com, Tel: 86-13524888984; JI Tong, Email: jitong70@hotmail.com, Tel: 86-13651658767

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【作者简介】 周文凯，医师，硕士，Email：cal310989@sjtu.edu.cn

【通信作者】 曹巍，副主任医师，博士，Email：caowei561521@hotmail.com，Tel：86-13524888984；共同通信作者，季彤，教授，博士，Email：jitong70@hotmail.com，Tel：86-13651658767

【**Abstract**】 **Objective** To investigate the role of long non-coding RNA double homeobox A pseudogene 9 (DUXAP9) in head and neck squamous cell carcinoma (HNSCC) and to evaluate the expression level, molecular function and mechanism of DUXAP9 in HNSCC cells. **Methods** Differential expression of lncRNAs between normal and tumor tissues in HNSCC tissues were screened using lncRNA microarray, the expression level of DUXAP9 in HNSCC tissues and its relationship with prognosis were analyzed in the TCGA database. The expression levels of DUXAP9 in HNSCC tissues and cell lines were detected using qRT-PCR. The function in HNSCC cells after DUXAP9 silencing was evaluated using the CCK-8 assay, wound healing assay, Transwell migration assay and subcutaneous xenograft assay in nude mice. Changes in the transcription and translation of epithelial-mesenchymal transition (EMT)-related proteins in head and neck squamous cell carcinoma cells after DUXAP9 silencing were detected using qRT-PCR and Western blot. **Results** lncRNA microarray results showed that, compared to adjacent normal tissues, DUXAP9 was abnormally upregulated in HNSCC tissues. Analysis from TCGA database showed that, compared to HNSCC patients with low DUXAP9 expression, HNSCC patients with high DUXAP9 expression had poorer survival. The relative expression of DUXAP9 in HNSCC tissues and 4 HNSCC cell lines increased compared to paired adjacent normal tissues as detected using qRT-PCR. Silencing DUXAP9 significantly inhibited the proliferation, migration and expression of EMT-related genes in HNSCC cells. The silencing of DUXAP9 significantly inhibited subcutaneous tumorigenesis of the HNSCC cell line CAL27 in nude mice. **Conclusion** Silencing DUXAP9 significantly inhibited the proliferation of HNSCC cells and subcutaneous xenografts in nude mice. DUXAP9 may mediate the migration of head and neck squamous cell carcinoma cells via the EMT pathway.

【**Key words**】 long non-coding RNA; double homeobox A pseudogene 9; gene silence; head and neck squamous cell carcinoma; epithelial mesenchymal transformation; E-cadherin; N-cadherin; Vimentin; cell proliferation; cell migration; subcutaneous xenograft in nude mice

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头颈鳞癌(head and neck squamous cell carcinoma,HNSCC)是世界第六大恶性肿瘤,2018年,头颈鳞癌新增89万病例,死亡造成45万人死亡[1-2]。大多数头颈鳞癌病例发生在口腔、咽和喉的上皮黏膜,严重限制了患者的咀嚼能力、言语、美容甚至危及生命[3]。头颈鳞癌发病风险因素有很多,包括吸烟、饮酒、咀嚼槟榔、人乳头状瘤病毒(human papillomavirus,HPV)病毒、口腔卫生状况差等[4-5]。其他风险因素还包括遗传因素、环境污染、免疫抑制等[4,6-7]。早期头颈鳞癌主要是采取手术或放射治疗,而对于中晚期疾病,临床上建议多学科治疗,不仅是为了提高生存率,也是为了提高患者的生活质量[8-9]。尽管如此,肿瘤转移、肿瘤复发和肿瘤耐药仍然是导致头颈鳞癌5年生存率差(低于60%)的主要原因[6,10-11]。筛选并验证肿瘤转移、复发和耐药相关的靶点,并基于此开发新的治疗策略,对延长晚期头颈鳞癌患者的生存时间有重要意义。

研究发现,非编码RNA(non-coding RNA)调控肿瘤的发生和进展,部分非编码RNA与肿瘤的转移、局部复发和临床预后密切相关[12-13]。长链非编码RNA(long non-coding RNA,LncRNA)作为非编码RNA的一种,参与细胞生理病理的过程[14],在包括肿瘤在内的疾病发生、发展中起着重要作用[15]。因此,阐明LncRNA生物学功能,有助于进一步揭示口腔鳞癌分子发病机制,为口腔鳞癌早期诊断、预后判断以及治疗提供有效靶点。DUXAP9是一种新发现的假基因来源的lncRNA[16],属于DUXA同源盒基因家族。同源盒基因编码DNA结合蛋白,其中许多蛋白被认为与早期胚胎发育有关[17]。同源盒基因编码60~63个氨基酸的DNA结合结构域,称为同源结构域[18]。早期研究表明,DUXAP9在肝细胞癌中异常高表达,其表达水平与患者的预后不良密切相关[16,19]。此外,研究还发现DUXAP9能促进肾癌细胞的生长和增殖[17]。目前DUXAP9在头颈鳞癌中功能和作用机制尚不清楚。本研究旨在探讨DUXAP9在头颈鳞癌组织和细胞中的表达,评估DUXAP9对头颈鳞癌细胞增殖、迁移和裸鼠皮下成瘤能力的影响,进一步探讨DUXAP9可能的分子作用机制。

1　材料和方法

1.1　组织标本、动物、细胞及试剂

30例新鲜头颈鳞癌及癌旁正常组织样本，均取自上海交通大学医学院附属第九人民医院口腔颌面头颈肿瘤科住院患者。本研究获得上海交通大学医学院附属第九人民医院伦理委员会的批准（沪九院科伦审：[2016]144号），所有参与者在入组前均签署知情同意书，并对应有完整的病理和临床资料。所使用的人源头颈鳞癌细胞系，包括WSU-HN4、WSU-HN6和WSU-HN30（以下分别称为HN4、HN6和HN30）均来自于美国国立卫生研究院，由美国马里兰大学牙学院友情馈赠；人源HNSCC细胞系CAL27购买于美国典型培养物保藏中心（American type culture collection，ATCC）；人正常鳞状上皮（normal oral keratinocytes，NOK）细胞取自临床拔牙后患者的正常牙龈黏膜组织进行原代培养。SPF级裸鼠（7周）购自上海交通大学医学院实验动物科学部。

OE Biotech Human WT lncRNA芯片（Affymetrix公司，美国），Trizol（15596018，Invitrogen公司，美国），Hiscript QRT supermix for qPCR（+gDNA WIPER）（R123-01，Vazyme公司，中国），2× SYBR Green qPCR Master Mix试剂盒（bimake公司，美国），Lipofectamine 3000试剂（Invitrogen公司，美国），DUXAP9 Smart Silencer（SS-DUXAP9）、SS-NC（锐博生物有限公司，中国）；GAPDH、DUXAP9、E-钙黏蛋白（E-cadherin）、N-钙黏蛋白（N-cadherin）、Snail、波形蛋白（Vimentin）的实时定量PCR引物合成（上海生工生物工程有限公司，中国）。

兔抗人多克隆E-cadherin抗体、N-cadherin抗体、Vimentin抗体、Snail抗体（Abclonal，中国），羊抗兔lgG二抗（Abclonal，中国）。LumiQ通用型ECL发光液（Sharebio，中国），1 × 无蛋白快速封闭液（雅酶，中国）。含0.02% EDTA的0.25%胰蛋白酶溶液（Gibco，美国），BCA蛋白浓度测定试剂盒（碧云天，中国）。CCK-8试剂（Dojindo，Kumamoto，日本），Transwell小室（Corning，美国），4%多聚甲醛（Servicebio，中国），0.1%结晶紫染液（Solarbio，中国）。胎牛血清（Fetal bovine serum，FBS）（Gibco公司，美国），青霉素和链霉素（Gibco公司，美国）、DMEM培养基（源培，中国）。CO_2恒温培养箱（Thermo Fisher，美国）。微量移液器（Eppendorf，德国），UV/Vis微板分光光度计（Multiskan™ Sky Spectrophotometer，Thermo Scientific，美国），酶标仪（Multiskan™ Sky High，ThermoFisher，美国），高速离心机（CT15RE，Hitachi，日本），倒置相差显微镜（GFM-600，上海光密仪器有限公司，中国），高速台式离心机（Multifuge X1 X1R，ThermoFisher，美国），罗氏480荧光定量PCR仪（Roche，美国），化学发光成像仪（ImageQuant LAS 4000 mini，General Electric Company，美国）。

1.2　方法

1.2.1　头颈鳞癌组织差异表达的lncRNA的筛选　使用OE Biotech Human WT lncRNA芯片筛选5对头颈鳞癌组织及其配对的癌旁正常组织中差异表达的lncRNA。筛选标准为Log2 Fold Change > 1.5，且$P < 0.05$，筛选Kaplan Meier预后分析存在显著直接相关性（HR大于1.4，且$P < 0.001$）的lncRNA。

1.2.2　TCGA数据库中分析DUXAP9在头颈鳞癌组织中的表达水平及其与临床预后的关系　首先进行泛癌分析，头颈鳞癌患者（502例，工作流类型：HTseq-FPKM）的基因表达数据及相应的临床信息从TCGA的头颈鳞癌项目（https://genomecancer.ucsc.edu/）下载。纳入诊断为头颈鳞癌且随访信息完整的患者。之后将level 3 HTseq-FPKM数据转换为TPM（transcripts per million）格式并进行log2转化，以进行进一步分析。不可用和未知的临床特征被认为是缺失值。本研究符合TCGA规定的出版指南。

统计分析采用R（3.6.3）进行统计分析。采用Kaplan-Meier方法分析DUXAP9表达与总生存期（overall survival，OS）相关的临床病理特征。用Wilcoxon秩和检验比较DUXAP9在肿瘤组织和正常组织中的表达。将样本分为高表达组和低表达组（中位数DUXAP9表达水平作为截断值）。接下来，使用SurvivalROC R软件包绘制ROC曲线，测试DUXAP9作为诊断标志物的性能。使用ggplot2 R包用于DUXAP9和Snail的表达相关性分析和可视化。

1.2.3　qRT-PCR检测头颈鳞癌组织样本和细胞系中DUXAP9表达　根据Trizol操作说明书对20例组织样本的匀浆和HN4、HN6、HN30、CAL27、NOK细胞分别进行总RNA抽提；根据Hiscript QRT supermix for qPCR（+gDNA WIPER）操作说明书进行RNA逆转录获得cDNA。2 × SYBR Green qPCR Master Mix试剂盒进行qRT-PCR检测及数据分析。引物列表如表1所示。

表1　PCR引物序列

Table 1　Sequences of PCR primers

Primers	Sequences
DUXAP9	Forward: TGGCTGGTGGAGGATGTCTT
	Reverse: CCTGGGCTCCCTCAAATCAG
E-cadherin	Forward: CGAGAGCTACACGTTCACGG
	Reverse: GGGTGTCGAGGGAAAAATAGG
N-cadherin	Forward: TGCGGTACAGTGTAACTGGG
	Reverse: GAAACCGGGCTATCTGCTCG
Snail	Forward: TCGGAAGCCTAACTACAGCGA
	Reverse: AGATGAGCATTGGCAGCGAG
GAPDH	Forward: GAACGGGAAGCTCACTGG
	Reverse: GCCTGCTTCACCACCTTCT
Vimentin	Forward: AGTCCACTGAGTACCGGAGAC
	Reverse: CATTTCACGCATCTGGCGTTC

1.2.4　细胞转染　沉默DUXAP9的Smart Silencer（SS-DUXAP9）由锐博生物有限公司设计和合成。siRNA#1：GATAGAATAGTGACAATAA；siRNA#2：GACCCATCACAAAGTTTAA；siRNA#3：GAGATATGTAGTAAAGCAA；ASO#1：GCTGTACACAAATACTGAAC；ASO#2：TACAATCTAAGTGGTTGGAC；ASO#3：AAATATGCACTTCCCACAAC。按照制造商的说明，使用Lipofectamine 3000试剂对CAL27细胞和HN6细胞转染相应的siRNA和ASO。

1.2.5　qRT-PCR检测转染SS-DUXAP9的CAL27和HN6细胞中DUXAP9相对表达量　对CAL27细胞和HN6细胞转染SS-NC（乱序siRNA和ASO对照）或SS-DUXAP9，24 h后提取总RNA后进行qRT-PCR实验检测DUXAP9的表达，用GAPDH基因作为对比的内参基因。

1.2.6　细胞划痕、Transwell迁移、CCK-8实验　①细胞划痕实验　比较转染SS-NC（对照组）或SS-DUXAP9（实验组）的CAL27细胞和HN6细胞在培养0 h和24 h细胞迁移能力的差异，以验证DUXAP9的沉默是否会对CAL27和HN6细胞的迁移能力有影响，标记并测量三个视野，实验重复3次。

②Transwell迁移实验　将转染SS-NC或SS-DUXAP9 24 h后的CAL27细胞和HN6细胞，4×10^4个细胞用200 μL无血清培养基重悬接种于24孔板的8 μm孔隙率的Transwell小室上室，下室加入600 μL的DMEM+20%胎牛血清。24～36 h后，穿过小室的细胞用4%多聚甲醛室温固定15 min，0.1%结晶紫染色30 min。显微镜拍摄Transwell上室底部细胞，Image J软件进行计数。

③CCK-8实验　将转染SS-NC或SS-DUXAP9 24 h后的CAL27细胞和HN6细胞以1 000个/孔的密度接种到96孔板中，重复3个副孔。将10 μL CCK-8试剂添加到90 μL培养基中。随后将细胞在37 ℃下培养2 h，并使用UV/Vis微板分光光度计在450 nm和600 nm处测量吸光度。

1.2.7　qRT-PCR、Western blot检测EMT相关基因、蛋白表达　将转染SS-NC或SS-DUXAP9 48 h后的CAL27细胞和HN6细胞进行总RNA的提取，进行qRT-PCR实验，检测GAPDH、DUXAP9、E-cadherin、N-cadherin、Snail和Vimentin的RNA表达水平，操作同1.2.5。

将转染SS-NC或SS-DUXAP9 72 h后的CAL27细胞和HN6细胞进行蛋白抽提，制胶上样电泳，免疫印迹（湿转），封闭40 min，加入E-cadherin、Vimentin、N-cadherin、Snail和GAPDH抗体一抗，4 ℃过夜孵育。TBST洗膜3次，10 min/次。加入二抗孵育1 h。TBST洗膜3次，10 min/次。ECL试剂盒显色，化学发光成像仪进行化学发光，保存图片分析。

1.2.8　裸鼠皮下成瘤实验　SPF级裸鼠（7周）购自上海交通大学医学院实验动物科学部。在实验前，这些动物被饲养在（22 ± 1）℃和（50 ± 5）%湿度的SPF标准笼子中。

小鼠分对照组（注射转染SS-NC的CAL27细胞皮下成瘤组）和实验组（注射转染SS-DUXAP9的CAL27细胞皮下成瘤组），每组5只小鼠。将1×10^6个CAL27细胞接种于5只小鼠左右背侧皮下，注射100 μL无血清DMEM培养基含1×10^6个预处理的CAL27细胞。每4 d用卡尺测量肿瘤大小。肿瘤体积的测量方法如下：肿瘤体积 = 长 × 宽 × 宽/2。动物处死后，采集肿瘤标本，测量重量。所有的动物实验的操作和处理得到上海交通大学医学院附属第九人民医院伦理审查委员会的批准（伦理审批号：SH9H-2019-A56-1）。

1.3　统计学分析

使用R（3.6.3）进行统计学分析。TCGA数据库相关统计学分析见1.2.2。采用单因素方差分析或Student t检验分析各组间差异的显著性。$P < 0.05$为差异有统计学意义。

2　结　果

2.1　DUXAP9在头颈鳞癌组织样本和细胞系中高表达

对5对头颈鳞癌患者组织样本和配对的癌旁组织样本进行lncRNA转录组测序分析后发现，与

癌旁组织相比，DUXAP9在头颈鳞癌中异常高表达（图1a、1b）。进一步为了评估DUXAP9在多种癌症中的水平，首先通过TCGA数据库进行泛癌分析（包括肿瘤组织和癌旁组织），发现DUXAP9在大多数恶性肿瘤类型中表达上调，包括头颈鳞癌，DUXAP9在TCGA正常标本与TCGA肿瘤标本中的表达有统计学差异（图1c）。接下来，生存曲线分析发现，与DUXAP9低表达患者相比，DUXAP9高表达的患者生存率差（图1d）。此外，利用TCGA数据库头颈鳞癌数据进行ROC分析显示，以DUXAP9的表达量可作为标准区分肿瘤及正常组织，其AUC值可达0.884，DUXAP9可以作为头颈鳞癌患者早期诊断的生物标志物（图1e）。进一步为评估DUXAP9在头颈鳞癌中的表达水平，在头颈鳞癌组织样本（图1f）和细胞系（图1g）中检测DUXAP9的表达水平，qRT-PCR实验表明DUXAP9在头颈鳞癌组织标本和细胞系中异常高表达。

2.2　沉默DUXAP9能显著抑制头颈鳞癌细胞的增殖和迁移能力

SS-DUXAP9能有效地抑制DUXAP9在CAL27和HN6细胞中的表达水平（图2a），细胞划痕实验显示，与对照组相比，沉默DUXAP9能显著抑制CAL27细胞和HN6细胞的迁移能力（图2b），Transwell迁移实验也进一步证实沉默DUXAP9能显著抑制CAL27和HN6细胞的迁移能力（图2c）。同时，CCK-8实验结果表明沉默DUXAP9可以显著抑制CAL27和HN6细胞的增殖能力（图2d）。

2.3　沉默DUXAP9能显著抑制EMT相关基因的表达

经qRT-PCR实验证实，在CAL27和HN6细胞中，沉默DUXAP9基因能显著增加E-cadherin的mRNA表达水平，降低N-cadherin、Vimentin和Snail的mRNA表达水平（图3a、3b）；Western blot实验进一步证实，在CAL27和HN6细胞中，沉默DUXAP9基因也能显著增加E-cadherin的蛋白表达水平，降低N-cadherin、Vimentin和Snail蛋白表达水平（图3c）。此外，TCGA数据库数据进一步提示，在头颈鳞癌组织样本中，DUXAP9与Snail mRNA的表达水平呈正相关性（$R=0.28$）（图3d）。

2.4　沉默DUXAP9可明显抑制裸鼠皮下成瘤能力

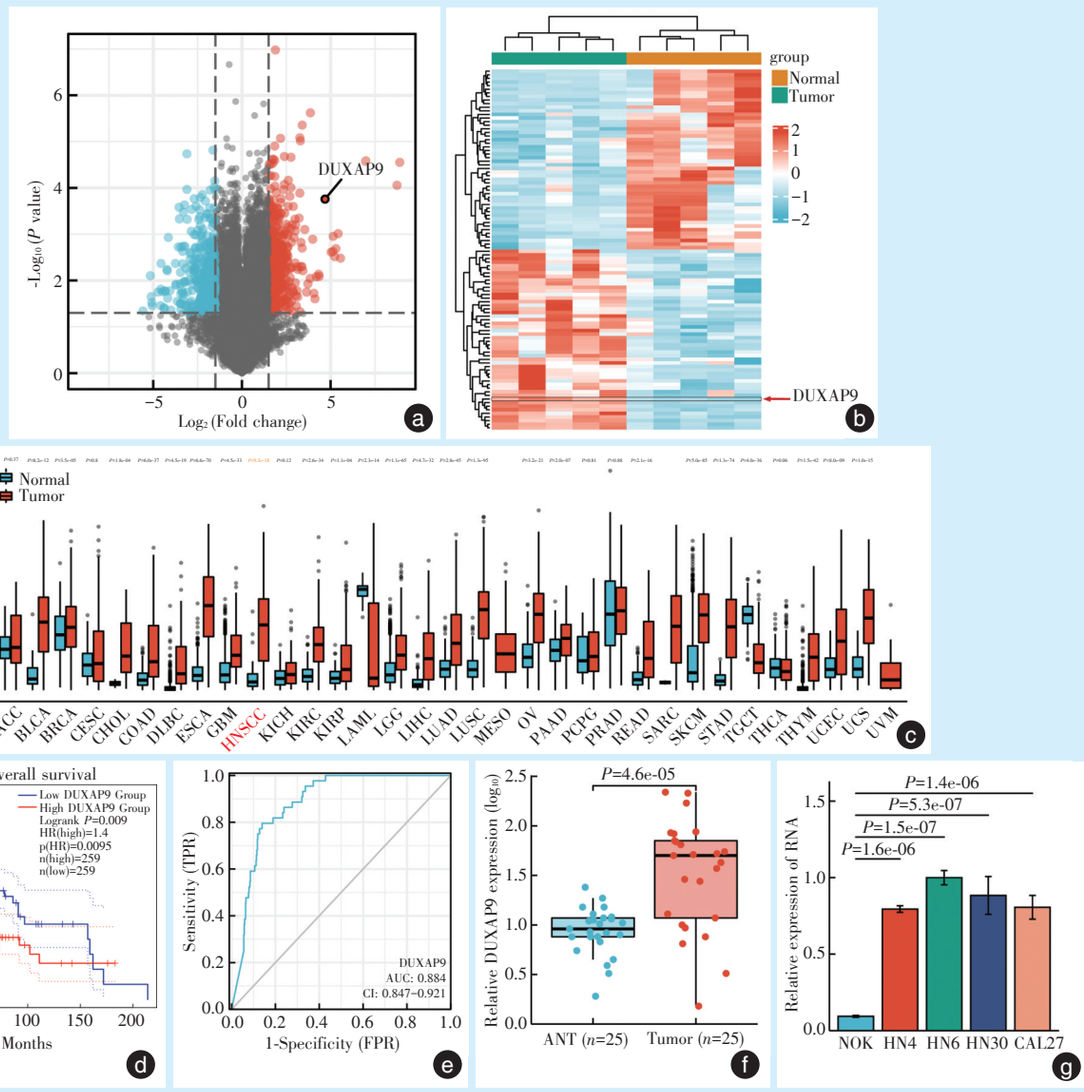
裸鼠皮下成瘤实验表明，沉默DUXAP9可显著抑制CAL27移植瘤的生长；与对照组相比，沉默组的肿瘤体积和重量显著减少（图4）。

3　讨　论

越来越多的证据表明，lncRNAs在人类疾病进展以及各种癌症的发生和发展中起着重要作用[20-22]。研究发现，异常表达的lncRNAs参与了头颈鳞癌细胞的一些生物学过程，如增殖、分化、侵袭和转移[23]。有报道，lncRNA DUXAP9可以直接与Cbl-b（Cbl proto-oncogene B）结合，增强表皮生长因子受体（epidermal growth factor receptor，EGFR）信号通路，促进非小细胞肺癌进展[24]。在肾癌细胞中，DUXAP9可以发生N6-腺苷甲基化修饰，并与胰岛素样生长因子2 mRNA结合蛋白2（insulin like growth factor 2 mRNA binding protein 2，IGF2BP2）结合增加其稳定性，通过PI3K/AKT通路促进肾癌细胞的增殖和迁移能力[25]。在肝细胞癌中，DUXAP9直接与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）的3′非翻译区（UTR）结合，增强了SOX9 mRNA的稳定性，增加了SOX9的表达，从而促进肝细胞癌的进展[26]。本课题组通过lncRNA转录组学比较5对头颈鳞癌组织标本和配对癌旁组织标本的差异表达lncRNAs，发现DUXAP9在头颈鳞癌组织中表达显著上调，通过TCGA数据库分析，DUXAP9上调表达与头颈鳞癌患者预后差密切相关；裸鼠皮下成瘤实验表明，沉默DUXAP9可显著抑制CAL27移植瘤的生长。

肿瘤的转移是癌症死亡的主要原因，在癌症中，EMT（epithelial-mesenchymal transition）通过增强癌细胞的迁移能力、侵袭能力和对凋亡刺激的抵抗能力，赋予癌细胞转移特性[27]，EMT是由一系列复杂的生物和生化变化组成[28]，这些变化导致细胞失去分化的上皮细胞样形态，而获得更多的间叶细胞样的表型[29]。Snail可通过EMT在头颈鳞癌中诱导和维持肿瘤干细胞样特性的作用[30]。研究证明，NBS1可通过上调Snail来诱导EMT的表型和促进头颈鳞癌的迁移侵袭能力[31]。本研究通过对TCGA数据库数据分析的结果显示，在头颈鳞癌组织样本中，DUXAP9与Snail mRNA的表达水平呈正相关性，提示DUXAP9可能通过上调Snail的表达来促进EMT进程。

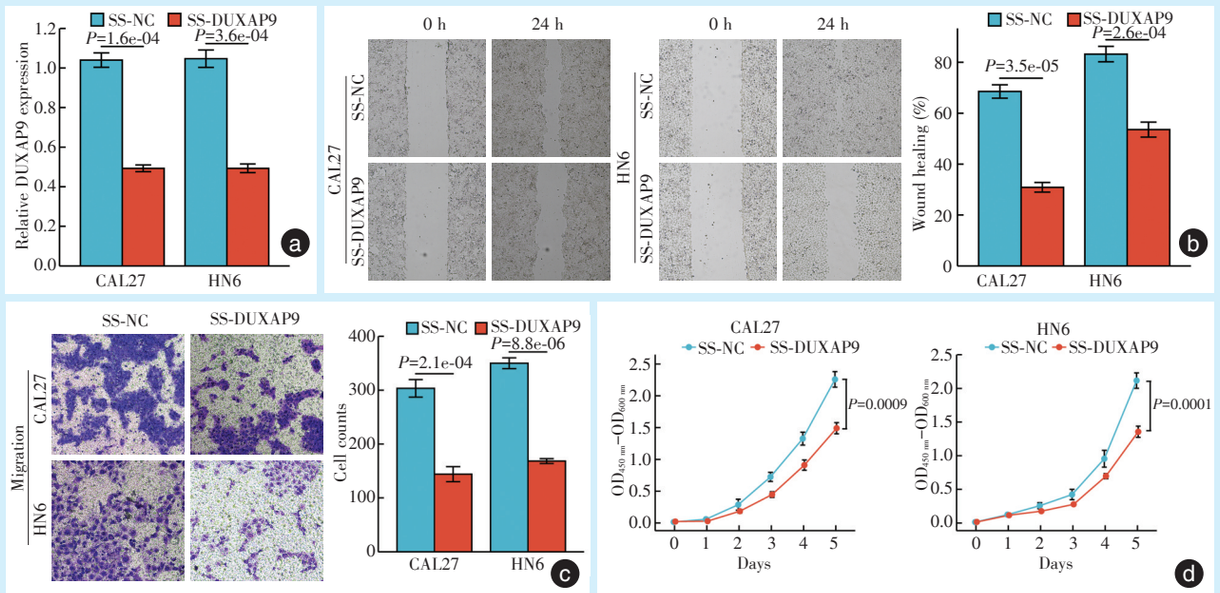
研究表明，高表达的N-cadherin和低表达的E-cadherin与鳞状细胞癌的组织学分化、侵袭模式和淋巴结转移密切相关[32-33]。而上皮-间充质转化的特点是EMT的标志蛋白如N-cadherin、Vimentin等上调和E-cadherin等下调，这一过程受到复杂的信



a: the volcano plots used to visualize the differential expression of lncRNAs between normal and tumor tissues; b: DUXAP9 was upregulated in HNSCC tissues using lncRNA microarray and hierarchical clustering analysis [fold change(FC) > 1.5, $P < 0.05$]; c: DUXAP9 expression in pan-cancer from normal TCGA samples of GTEx combined with samples of TCGA HNSCC; d: Kaplan-Meier curves of overall survival with different expression levels of DUXAP9 in HNSCC patients; e: ROC analysis of DUXAP9 expression showing promising discrimination power between tumor and normal tissues; f: the relative expression of DUXAP9 in HNSCC tissues increased compared to paired adjacent normal tissues as detected using qRT-PCR; g: the relative expression of DUXAP9 in 4 HNSCC cell lines increased compared to NOK cells as detected using qRT-PCR. DUXAP9: double homeobox A pseudogene 9; HNSCC: head and neck squamous cell carcinoma; TCGA: the Cancer Genome Atlas; GTEx: the genotype-tissue expression; ROC: receiver operating characteristic; TPM: transcripts per million; ACC: adrenocortical carcinoma; BLCA: bladder urothelial carcinoma; BRCA: breast invasive carcinoma; CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: cholangiocarcinoma; COAD: colon adenocarcinoma; DLBC: lymphoid neoplasm diffuse large B-cell lymphoma; ESCA: esophageal carcinoma; GBM: glioblastoma multiforme; KICH: kidney chromophobe; KIRC: kidney renal clear cell carcinoma; KIRP: kidney renal papillary cell carcinoma; LAML: acute myeloid leukemia; LGG: brain lower grade glioma; LIHC: liver hepatocellular carcinoma; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; MESO: mesothelioma; OV: ovarian serous cystadenocarcinoma; PAAD: pancreatic adenocarcinoma; PCPG: pheochromocytoma and paraganglioma; PRAD: prostate adenocarcinoma; READ: rectum adenocarcinoma; SARC: sarcoma; SKCM: skin cutaneous melanoma; STAD: stomach adenocarcinoma; TGCT: testicular germ cell tumors; THCA: thyroid carcinoma; THYM: thymoma; UCEC: uterine corpus endometrial carcinoma; UCS: uterine carcinosarcoma; UVM: uveal melanoma; TPR: true positive rate; FPR: false positive rate; ANT: adjacent normal tissues; NOK: normal oral primary keratinocytes

Figure 1　Expression level and significance of DUXAP9 in head and neck squamous cell carcinoma

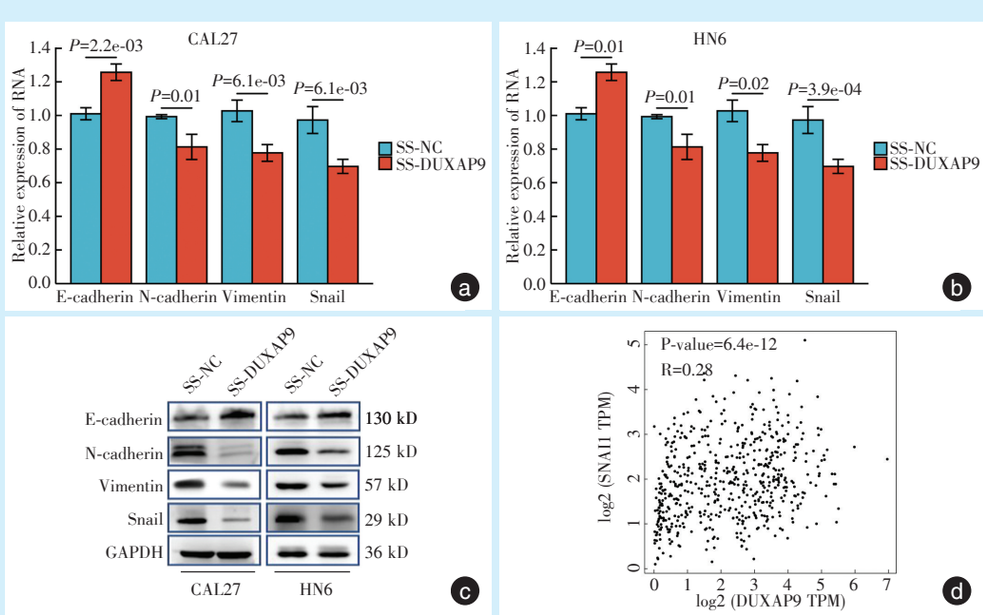
图1　DUXAP9在头颈鳞癌中的表达水平及意义



a: the relative expression of DUXAP9 in CAL27 and HN6 cells transfected with SS-DUXAP9 was detected using qRT-PCR; b: the migration behavior of CAL27 and HN6 cells transfected with SS-DUXAP9 was assessed using wound healing assays; c: the cell migration abilities of CAL27 and HN6 cells transfected with SS-DUXAP9 were determined using Transwell assays; d: the effect of DUXAP9 on cell proliferation was evaluated in CAL27 and HN6 cells transfected with SS-DUXAP9 using CCK-8 assays. HNSCC: head and neck squamous cell carcinoma; SS-DUXAP9: DUXAP9 Smart Silencer

Figure 2　Silencing DUXAP9 significantly inhibited HNSCC cell proliferation and migration *in vitro*

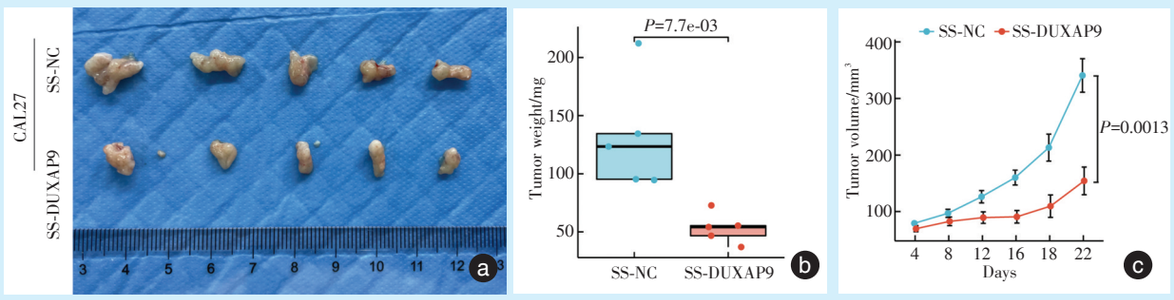
图2　沉默DUXAP9能显著抑制头颈鳞癌细胞的增殖和迁移表型



a&b: the mRNA expression of E-cadherin, N-cadherin, vimentin, and Snail was detected using qRT-PCR when DUXAP9 was knocked down in CAL27 and HN6 cells; c: the protein expression of E-cadherin, N-cadherin, vimentin, and Snail was detected using Western blot when DUXAP9 was knocked down in CAL27 and HN6 cells; d: the expression correlation of DUXAP9 and Snail in the TCGA database; TPM: transcripts per million; EMT: epithelial-mesenchymal transition; DUXAP9: double homeobox A pseudogene 9

Figure 3　Silencing DUXAP9 significantly inhibited the expression of EMT-related genes

图3　沉默DUXAP9能显著抑制EMT相关基因的表达



The volumes and weights of tumors from CAL27 cells transduced with SS-DUXAP9 or SS-NC tumor-bearing nude mice are shown, n=5/group.
HNSCC: head and neck squamous cell carcinoma; DUXAP9: double homeobox A pseudogene 9

Figure 4　Silencing DUXAP9 significantly inhibited subcutaneous tumorigenesis of the HNSCC cell line CAL27 in nude mice

图4　沉默DUXAP9能显著抑制头颈鳞癌细胞CAL27的裸鼠皮下成瘤能力

号通路和转录因子网络的严格调控[27]，研究显示，Snail可在头颈鳞癌细胞和口腔上皮细胞中驱动EMT，下调上皮粘附物如E-cadherin和β-catenin，和诱导间充质标记物如N-cadherin的上调，进而影响肿瘤的增殖转移能力[34]。AKT诱导的lncRNA VAL通过减少trim16依赖的Vimentin降解，促进肿瘤的EMT进展[22]。LncRNA HOTAIR可通过招募EZH2和H3K27me3到局部染色质中，从而抑制E-cadherin的表达来促进OSCC的恶化[19]。本研究结果提示，沉默DUXAP9能显著增加E-cadherin的mRNA和蛋白表达水平，降低N-cadherin、Vimentin和Snail的mRNA和蛋白表达水平，提示DUXAP9可能通过调控EMT介导的头颈鳞癌细胞的迁移。

综上所述，DUXAP9可以促进头颈鳞癌的增殖、迁移和裸鼠皮下成瘤能力。

【Author contributions】 Cao W, Ji T conceived and designed the study. Zhou WK, Wang JX conducted the *in vitro* experiments. Wang YF, Chen M conducted the *in vivo* experiments. Zhou WK, Liu ZQ wrote the manuscript. Zhang X, Tao XR analyzed the data. All authors have read and approved the final manuscript.

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