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· 基础研究 ·

FKBP10在口腔鳞状细胞癌中的表达及功能研究

方志凯, 金辉, 杨姗, 蒋男, 张茗宇, 周爽, 李畅, 李丽丽

哈尔滨医科大学附属第一医院儿童口腔科, 黑龙江 哈尔滨 (150001)

【摘要】 目的 探讨FK506结合蛋白10(FK506 binding protein 10, FKBP10)在口腔鳞状细胞癌(oral squamous cell carcinoma, OSCC)中的表达和功能作用, 为OSCC的预后判断和靶向治疗提供依据。方法 在癌症基因组图谱(the cancer genome atlas, TCGA)数据库中选择OSCC样本284例, 正常样本19例, 并进行mRNA表达分析和诊断分析。在基因表达图谱交互分析网站上进行FKBP10与癌症预后的生存分析。通过实时荧光定量PCR和Western Blot检测4种OSCC细胞系及SAS和SCC9细胞转染siRNA后FKBP10的mRNA和蛋白表达情况。通过CCK8法检测FKBP10沉默后的细胞增殖能力, 同时使用流式细胞仪检测细胞周期分布及细胞凋亡。通过伤口愈合实验和侵袭实验检测细胞迁移和侵袭能力。通过蛋白组学和Western Blot分析FKBP10沉默后的总蛋白和磷脂酰肌醇3激酶(phosphatidylinositol 3-kinase, PI3K)-丝氨酸/苏氨酸激酶(protein kinase B, AKT)的表达变化。结果 根据基因表达量分析结果显示, FKBP10的mRNA表达水平在OSCC中的表达显著高于正常组织($P < 0.001$)。在诊断方面, FKBP10的表达量对OSCC具有一定的诊断价值($P < 0.05$)。FKBP10与OSCC的生存分析表明, FKBP10的高表达导致患者生存期下降与预后不良($P < 0.05$)。OSCC细胞系中FKBP10的mRNA和蛋白表达高于正常口腔角质形成细胞($P < 0.001$)。沉默FKBP10可降低SAS和SCC9细胞的增殖、侵袭和迁移能力($P < 0.001$), 细胞周期被阻滞在G0/G1期($P < 0.001$), 凋亡显著上升($P < 0.05$)。蛋白质质谱分析及Western Blot显示, FKBP10沉默显著下调Ras相关蛋白1(ras-related protein, RAP1)信号通路中以RAP鸟嘌呤核苷酸交换因子1(rap guanine nucleotide exchange factor 1, RAPGEF1)为主的多种蛋白的表达($P < 0.05$)和PI3K-AKT蛋白的磷酸化($P < 0.05$)。结论 FKBP10在OSCC中高表达, 导致患者预后不良。FKBP10表达下调可通过RAP1-PI3K-AKT轴抑制OSCC细胞的增殖、迁移和侵袭, 阻碍细胞周期进程并促进凋亡。FKBP10是OSCC的潜在治疗靶点和预后生物标志物。

【关键词】 FK506结合蛋白10; 口腔鳞状细胞癌; 生物信息学分析; 细胞增殖; 细胞周期; 细胞侵袭; 细胞迁移; 预后

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Expression and functional study of FKBP10 in oral squamous cell carcinoma FANG Zhikai, JIN Hui, YANG Shan, JIANG Nan, ZHANG Mingyu, ZHOU Shuang, LI Chang, LI Lili. Department of Pediatric Dentistry, The First Affiliated Hospital of Harbin Medical University, Harbin 150001, China

Corresponding author: LI Lili, Email: iamlilili@126.com

【Abstract】 Objective To investigate the expression and functional role of FK506 binding protein 10 (FKBP10) in oral squamous cell carcinoma (OSCC), and to provide a research basis for the estimated prognosis and targeted therapy of OSCC. **Methods** A total of 284 OSCC samples and 19 normal samples were selected from the Cancer Genome Atlas (TCGA) database, and diagnostic analysis was performed to determine mRNA expression. Survival analysis for FKBP10 and OSCC was conducted on a gene expression profile interaction analysis website. Real-time fluorescence



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【作者简介】 方志凯, 硕士研究生在读, Email: fangzhikai1998@163.com

【通信作者】 李丽丽, 副主任医师, 博士, Email: iamlilili@126.com

quantitative PCR and Western Blot were used to detect the mRNA and protein expression of FKBP10 in four OSCC cell lines and SAS and SCC9 cells transfected with siRNA. The cell proliferation ability of FKBP10-silenced cells was detected using the CCK8 method, and the cell cycle distribution and apoptosis were detected by flow cytometry. Cell migration and invasion ability were detected through wound healing and invasion experiments. The expression changes of total protein and phosphatidylinositol 3-kinase (PI3K)-serine/threonine kinase (AKT) after FKBP10 silencing were analyzed by proteomics and Western Blot. **Results** According to the analysis of gene expression levels, the mRNA expression level of FKBP10 in OSCC was significantly higher than that in normal tissues ($P < 0.001$). In terms of diagnosis, the expression level of FKBP10 has unique diagnostic value for OSCC ($P < 0.05$). The survival analysis of FKBP10 and OSCC showed that a high expression of FKBP10 led to a decrease in patient survival and poor prognosis ($P < 0.05$). The expression of FKBP10 mRNA and protein in OSCC cell lines was higher than that in normal oral keratinocytes ($P < 0.001$). Silencing FKBP10 can reduce the proliferation, invasion, and migration ability of SAS and SCC9 ($P < 0.001$), and also block their cell cycle in the G0/G1 phase ($P < 0.001$), with a significant increase in apoptosis ($P < 0.05$). Protein mass spectrometry and Western blot analysis revealed that FKBP10 silencing significantly downregulated the expression of multiple proteins in the RAP1 signaling pathway, mainly RAP guanine nucleotide exchange factor 1 (RAPGEF1) ($P < 0.05$) and the phosphorylation of PI3K-AKT proteins ($P < 0.05$). **Conclusion** FKBP10 is highly expressed in OSCC, leading to poor prognosis for patients. Downregulated FKBP10 expression can inhibit the proliferation, migration, and invasion ability of OSCC cells, hinder cell cycle progression, and promote apoptosis via the RAP1-PI3K-AKT axis. FKBP10 is a potential therapeutic target and prognostic biomarker for OSCC.

【Key words】 FK506 binding protein 10; oral squamous cell carcinoma; bioinformatics analysis; cell proliferation; cell cycle; cell invasion; cell migration; prognosis

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口腔鳞状细胞癌(oral squamous cell carcinoma, OSCC)作为头颈部恶性肿瘤的主要病理类型,其发病本质源于口腔黏膜上皮细胞的恶性转化过程^[1]。解剖学特征显示,头颈部存在丰富的血管与密集的淋巴管分布,不仅为肿瘤增殖提供了适宜的微环境,更提高了癌症通过血管和淋巴转移的风险^[2]。值得注意的是,尽管分子诊断技术和联合治疗策略对癌症的诊治方面取得显著进展,但OSCC患者以年龄标准化的5年生存率仍停滞于50%^[3]。因此,探索调控OSCC进展的分子机制对于开发新的治疗方法和提高5年生存期至关重要。

内质网应激触发后,内质网伴侣蛋白介导未折叠蛋白反应(unfolded protein reaction, UPR),通过调控蛋白质合成与降解维持细胞稳态^[4]。因此,癌细胞可凭借UPR机制影响肿瘤恶性进展^[5]和耐药性^[6]。FK506结合蛋白是一类与免疫抑制药物FK506结合的内质网伴侣蛋白^[7]。其中,FK506结合蛋白10(FK506 binding protein 10, FKBP10)具有多重生物学效用,如促进蛋白质折叠^[8],响应内质网应激压力产生自身水解并维持内质网稳态^[9-10]。

研究表明,FKBP10可促进胶质瘤等癌症进展并与预后不良相关^[11],如与核糖体互作调节蛋白质翻译以驱动癌症干细胞样特征^[12];调节乳酸脱氢酶A磷酸化功能,增强肾透明细胞癌对缺氧治疗的抵抗等^[13]。值得注意的是,FKBP10特异性表达于肺损伤和发育期肺组织,而在正常成人肺中缺失^[14],提示其可能特异性高表达于增殖活跃细胞。然而FKBP10在OSCC中的作用尚不清楚。

本研究旨在探寻FKBP10在OSCC中的表达模式,并通过体外实验初步确定FKBP10在OSCC发生发展中的重要作用,为OSCC的治疗和预后判断提供一种潜在的治疗靶点和生物标志物。

1 资料和方法

1.1 主要试剂与仪器

人OSCC细胞系(SAS、CAL27、SCC9和SCC15)和人口腔角质形成细胞(human oral keratinocytes, HOK)为哈尔滨医科大学口腔医学院馈赠。DMEM高糖细胞培养基(12100046, Gibco, 美国);胎牛血清(10270106, Gibco, 美国);青霉素-链霉素溶液

(BL505A, Biosharp, 中国); 0.25%-EDTA 胰蛋白酶消化液(C100C1, NCM, 中国); jetPRIME®转染试剂(0000003060, Polyplus, 法国); Trizol (SM192, Seven, 中国); First Strand cDNA Synthesis Kit (SM136, Seven, 中国); SYBR Green qPCR Master Mix II (SM143, Seven, 中国); 蛋白酶和磷酸酶抑制剂混合液(NCM, 中国); RIPA 裂解液(WB3100, NCM, 中国); 细胞计数试剂盒-8(C6005, NCM, 中国); 细胞周期检测试剂盒(AC12L544, Life-ilab, 中国); 基质胶(356234, Corning, 美国); 10 %快速凝胶试剂盒(PG112, Epizyme, 中国); 细胞培养小室(353097, Corning, 美国); 一抗兔抗人FKBP10抗体(12172-1-AP, Proteintech, 中国); 一抗兔抗人 β -actin抗体(AC026, ABclonal, 中国); 一抗鼠抗人AKT抗体(2920T, CST, 美国); 一抗兔抗人P-AKT抗体(4060T, CST, 美国); 一抗兔抗人PI3K抗体(T40115S, Abmart, 中国); 一抗兔抗人P-PI3K抗体(T40116S, Abmart, 中国); 二抗山羊抗小鼠IgG-HRP抗体(M21001S, Abmart, 中国); 二抗山羊抗兔IgG-HRP抗体(M21002S, Abmart, 中国); 10 × TBST缓冲液(G0004, Servicebio, 中国); ECL显影液(SQ201, Epizyme, 中国); 倒置光学显微镜(DMi1, Leica, 德国); 酶标仪(Synergy H1, Bio Tek, 美国); 细胞培养箱(310, Thermo Fisher, 美国); 流式细胞仪(Fortessa, BD, 美国); 实时荧光定量PCR仪(qTower³, Analytik Jena AG, 德国); 凝胶成像仪(ChemiDocTM, Bio-rad, 美国)。

1.2 生物信息学分析

从癌症基因组图谱数据库(the cancer genome atlas, TCGA)中下载并整理OSCC的相关数据,最终纳入OSCC样本284例,正常对照样本19例。借助R语言中的stats和car包通过Mann-Whitney *U*检验明确OSCC与正常组织中FKBP10的表达水平。同时,在基因表达图谱交互分析网站上以FKBP10表达水平的中位值定义高表达组和低表达组,并将其表达量与癌症患者的生存期限进行生存分析^[15]。利用pROC包,通过受试者工作特征曲线(receiver operating characteristic, ROC)对曲线下的面积进行分析,以此评估FKBP10在OSCC诊断中的价值。

1.3 OSCC细胞系培养、分组与转染

将SAS、CAL27、SCC9、SCC15和HOK细胞培养于T25培养瓶中。细胞培养基为含1 %青霉素-链霉素,10 %胎牛血清的完全培养基。所有细胞均

培养于37℃、5% CO₂的细胞培养箱中并定期换液。当细胞汇合度达到90%左右时,按比例将细胞接种至新的培养瓶中继续培养或孔板中进行实验。对SAS和SCC9细胞使用siRNA进行敲低,并分为Vehicle组(仅使用jetPRIME®转染试剂)、si-NC组(转染siRNA-nonsense)、si-FK1组(转染si-FKBP10 sequence-1)、si-FK2组(转染si-FKBP10 sequence-2),siRNA序列见表1。将处于对数生长期,状态良好的细胞以适当的细胞密度接种到6孔板中。当汇合度达到60%时,按照所述分组要求进行转染。首先,使用200 μ L jetPRIME®转染试剂Buffer稀释100 pmol siRNA,然后加入4 μ L jetPRIME®转染试剂并轻轻混匀。静置15 min后将其按分组加入2 mL待转染的细胞基本培养基中。6 h后将弃掉含有转染试剂的培养基并更换为新鲜培养基继续培养。24 ~ 48 h后收集RNA或蛋白质,以验证敲低效率或进行其他实验。

表1 siRNA序列

Table 1 siRNA sequences

siRNA	Sequences
si-NC	5'-UUCUCCGAACGUGUCACGUTT-3'
si-FK1	5'-CUGCCGUGCUAAUCUUAATT-3'
si-FK2	5'-CACACCUACAAUACCUAUUAUAATT-3'

si-NC: negative control sequence; si-FK1: targeting FKBP10 sequence 1; si-FK2: targeting FKBP10 sequence 2; FKBP10: FK506 binding protein 10

1.4 实时荧光定量PCR

细胞培养24 h后取出6孔板,每孔使用1 mL Trizol进行RNA提取。通过Nanodrop定量RNA浓度后,使用逆转录试剂盒在每个样品中取1 μ g总RNA合成cDNA。然后在PCR仪上,使用2 × SYBR Green qPCR Master Mix II通过qRT-PCR定量不同样品中mRNA的表达。最后,按之前描述的方法^[16],将 β -actin作为管家基因进行分析。引物序列见表2。

1.5 蛋白质印迹分析

转染结束48 h后,将浓度为1%蛋白酶和磷酸酶抑制剂混合液的RIPA裂解液加入不同组的样品中以裂解细胞。充分裂解并离心后从上清液中收集细胞总蛋白,使用Bradford法定量蛋白质浓度。然后在恒定电压下通过十二烷基硫酸钠-聚丙烯酰胺凝胶从每个样品中分离等量的蛋白质。使用0.2 μ m聚偏氟乙烯膜进行电转后,在室温下将

表 2 引物序列

Table 2 Primer sequences

Genes	Forward primer sequences (5'-3')	Reverse primer sequences (5'-3')
FKBP10	GTTACCTCGCATACACAC	CCTCTCCACACACAT
β -actin	CCACGAAACTACCTCAACTCCATC	AGTGATCCTCCTCCTCGATCCTGTC

FKBP10: FK506 binding protein 10

其置于含有 5% 脱脂牛奶 1 × 含吐温的 Tris 盐缓冲液 (tris buffered saline with tween, TBST) 中封闭 2 h。然后将膜置于含有靶蛋白一抗的 1 × TBST 中在 4 °C 下孵育过夜。封闭结束后,使用 1 × TBST 洗涤 3 次,然后在二抗在室温下孵育 2 h。最后,在 ChemiDoc™ 成像系统上进行化学发光检测,并通过 Image J 软件对成像数据定量分析。

1.6 体外细胞活力测定

将处于对数增殖期的 SAS 和 SCC9 细胞以适当的密度接种在 6 孔板中。转染 24 h 后,用胰蛋白酶消化不同组的 SAS 或 SCC9。然后按每孔 5 000 个细胞接种在 96 孔板上并在 37 °C 下孵育。在每个孔中加入 10 μ L CCK-8 检测溶液和 100 μ L 不含胎牛血清的基础培养基,孵育 1.5 h 后,在酶标仪上测量 1、2、3、4 d 时的 450 nm OD 吸光度。

1.7 伤口愈合试验

将 SAS 和 SCC9 以适当的密度接种在 6 孔板中。当转染后细胞融合度达到 90% 左右时,将培养基更换为无血清基础培养基,用 200/1 000 μ L 移液管尖端划出规则的间隙。划痕后立即拍照,24 h 后再次记录细胞位置。所有图像均通过 Leica 倒置显微镜从 3 个独立的划痕中获得并计算平均值,实验重复 3 次。

1.8 侵袭实验

首先,将稀释后的基质胶加入上室底部,在细胞培养箱中孵育 3 h,再用基础培养基水化 30 min。将含有 50 000 个 SAS 和 SCC9 细胞的 200 μ L 无血清基础培养基加入上室。然后将含有 10% 血清的 500 μ L 完全培养基加入下室。培养 48 h 后,使用多聚甲醛固定 30 min,结晶紫染色 30 min,然后置于显微镜下拍照记录。该实验独立重复 3 次,每次记录 3 个随机视图以统计穿膜的细胞数目,并计算平均值。

1.9 流式细胞术检测细胞周期

转染 48 h 后,通过适当消化收集 SAS 和 SCC9 细胞。然后用 PBS 洗涤 1 次,将细胞重新悬浮在 75% 乙醇溶液中置于 -20 °C 过夜。进行检测时,离心后在每个样品加入 500 μ L 染色缓冲液、25 μ L

20 × 碘化丙啶 (propidium iodide, PI) 染色和 10 μ L 50 × RNase,在室温下避光孵育 30 min。最后,通过流式细胞仪分析,并使用 Modfit 5.0 确定所有样本中每个细胞周期的细胞百分比。

1.10 流式细胞术检测细胞凋亡

转染 48 h 后,收获细胞并使用 1 × PBS 洗涤并离心 2 次。使用细胞凋亡检测试剂盒,首先加入 500 μ L 1 × Binding Buffer 重悬细胞,加入 5 μ L AnnexinV-FITC 混匀后,加入 5 μ L PI 再次混匀,室温避光反应 15 min。使用流式细胞仪检测并通过 Flow jo 进行数据分析。

1.11 蛋白质液相色谱质谱

收集转染敲低 FKBP10 的实验组和对照组 SAS 细胞和 SCC9 细胞后进行总蛋白质提取。在经消化切割蛋白为多肽片段后使用高效液相色谱技术进行分离并分级进入质谱电场进一步离子化,最后进行质谱检测。随后使用根据 Uniprot 蛋白质数据库关联数据并分析。

1.12 统计学分析

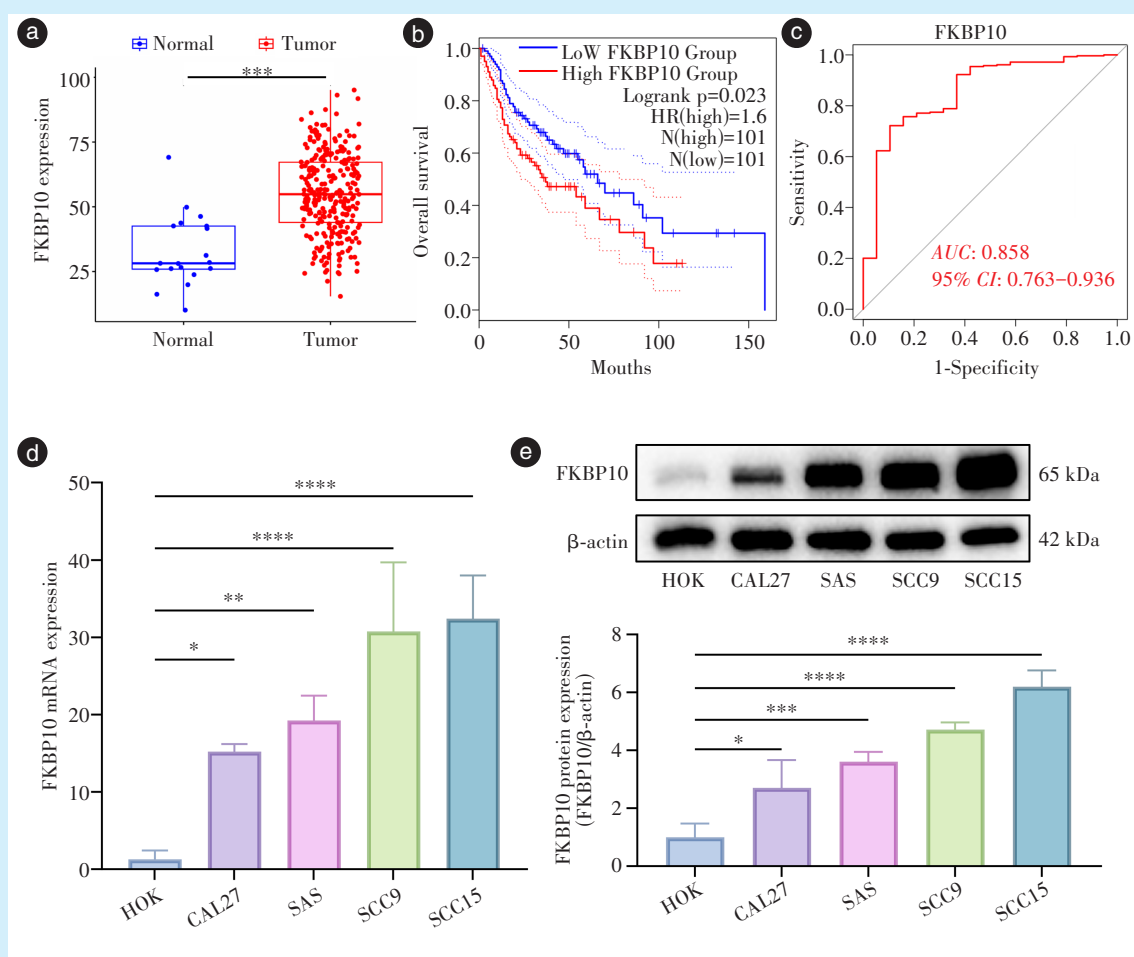
数据采用 GraphPad Prism 10.0 软件进行统计分析。所有数据均满足正态分布,多组间比较采用方差分析。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 FKBP10 在 OSCC 组织和细胞系中高表达并与预后不良相关

TCGA 数据分析结果表明 (图 1a), OSCC 组织中 FKBP10 的 mRNA 水平显著高于正常组织 ($P < 0.001$)。通过基因表达图谱交互分析网站对 OSCC 患者预后进行生存分析 (图 1b),发现 FKBP10 的表达与患者预后呈负相关性 ($P < 0.05$)。进一步基于 ROC 曲线对 FKBP10 在 OSCC 诊断中的价值进行分析,ROC 曲线下面积为 0.858,具有统计学意义 ($P < 0.05$,图 1c)。对 OSCC 细胞系的 qRT-PCR 和 Western Blot 结果显示 (图 1d、1e),在培养 48 h 后,FKBP10 在 OSCC 细胞 CAL27 ($P < 0.05$)、SAS ($P < 0.001$)、SCC9 ($P < 0.001$) 和 SCC15 ($P < 0.000 1$) 中

的 mRNA 和蛋白表达量均高于正常口腔角质形成细胞,差异具有统计学意义。



a: the mRNA expression of FKBP10 in OSCC was analyzed through The Cancer Genome Atlas database; b: the GEPIA2 online analysis tool was used to analyze the relationship between FKBP10 and the survival rate of patients with OSCC; c: ROC curve analysis was used to evaluate the value of FKBP10 in the diagnosis of OSCC; d: the expression of FKBP10 mRNA in OSCC cells and HOK; e: FKBP10 protein expression in OSCC cancer cells and HOK. OSCC: oral squamous cell carcinoma; HOK: human oral keratinocytes; FKBP10: FK506 binding protein 10. $n=3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

Figure 1 The correlation of FKBP10 expression with prognosis, and diagnostic analysis in OSCC, as well as its expression in cell lines

图1 FKBP10的表达与OSCC的预后和诊断分析的相关性及其在细胞系中的表达

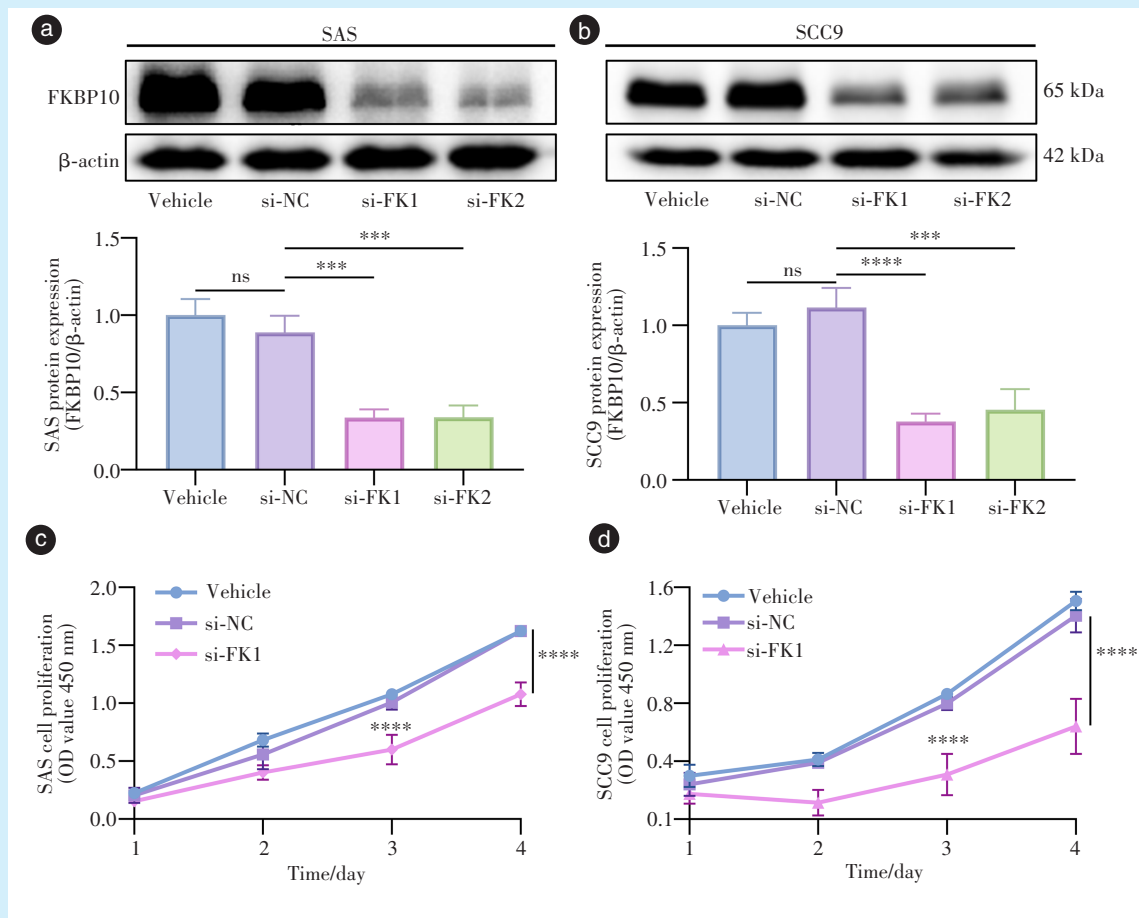
2.2 FKBP10 下调抑制 SAS 和 SCC9 细胞增殖、迁移和侵袭,促进细胞凋亡

如 Western Blot 结果所示(图 2a、2b), 6 h 转染结束后,培养至 48 h 后 SAS 和 SCC9 细胞中两种不同序列 siFKBP10 的蛋白沉默效率均达到 65% ($P < 0.001$),其中 si-FK1 组中 FKBP10 蛋白表达降低最为显著。因此,选择 si-FK1 组进行后续表型的研究。

CCK8 实验结果显示(图 2c、2d), si-FK1 组在 2 d ($P < 0.001$)、3 d ($P < 0.0001$)、4 d ($P < 0.0001$)时细胞的增殖能力受到抑制,差异有统计学意义;相

比之下, si-NC 组与 Vehicle 组的增殖差异无统计学意义 ($P > 0.05$), 这表明 si-FK1 组增殖抑制是受到 FKBP10 沉默后功能丧失并非转染试剂的毒性。

采用流式细胞术进一步探索两种细胞系的细胞周期变化(图 3a), 在转染 FKBP10 siRNA 的 48 h 后, 流式细胞仪检测两种细胞系均显示出约 10% 的 G0/G1 期阻滞 ($P < 0.001$)。在转染 siRNA 48 h 后, 流式细胞仪检测 FKBP10 的蛋白沉默导致 SAS 细胞和 SCC9 细胞趋向于早期凋亡 ($P < 0.01$), 并且其总体凋亡水平产生显著上升(图 3b)。



a & b: compared with the Vehicle group and si-NC group, the si-FKBP10 groups all showed reduced expression in SAS (a) and SCC9 (b) cells; c & d: in CCK-8 experiments, the proliferation rates at 1, 2, 3, and 4 days were lower in the si-FKBP10 group compared to the si-NC group in SAS (c) and SCC9 (d) cells. Vehicle group: only the transfection reagent group; si-NC group: negative control group; si-FK1 and si-FK2 groups: knockdown group using sequence 1 or 2; FKBP10: FK506 binding protein 10; OSCC: oral squamous cell carcinoma. $n=3$, ns: not significant, *** $P < 0.001$, **** $P < 0.0001$

Figure 2 Knockdown expression of FKBP10 in human OSCC cell line SAS and SCC9 and its effect on cell proliferation

图2 FKBP10在人OSCC细胞系SAS和SCC9细胞中敲低后的表达情况及对细胞增殖的影响

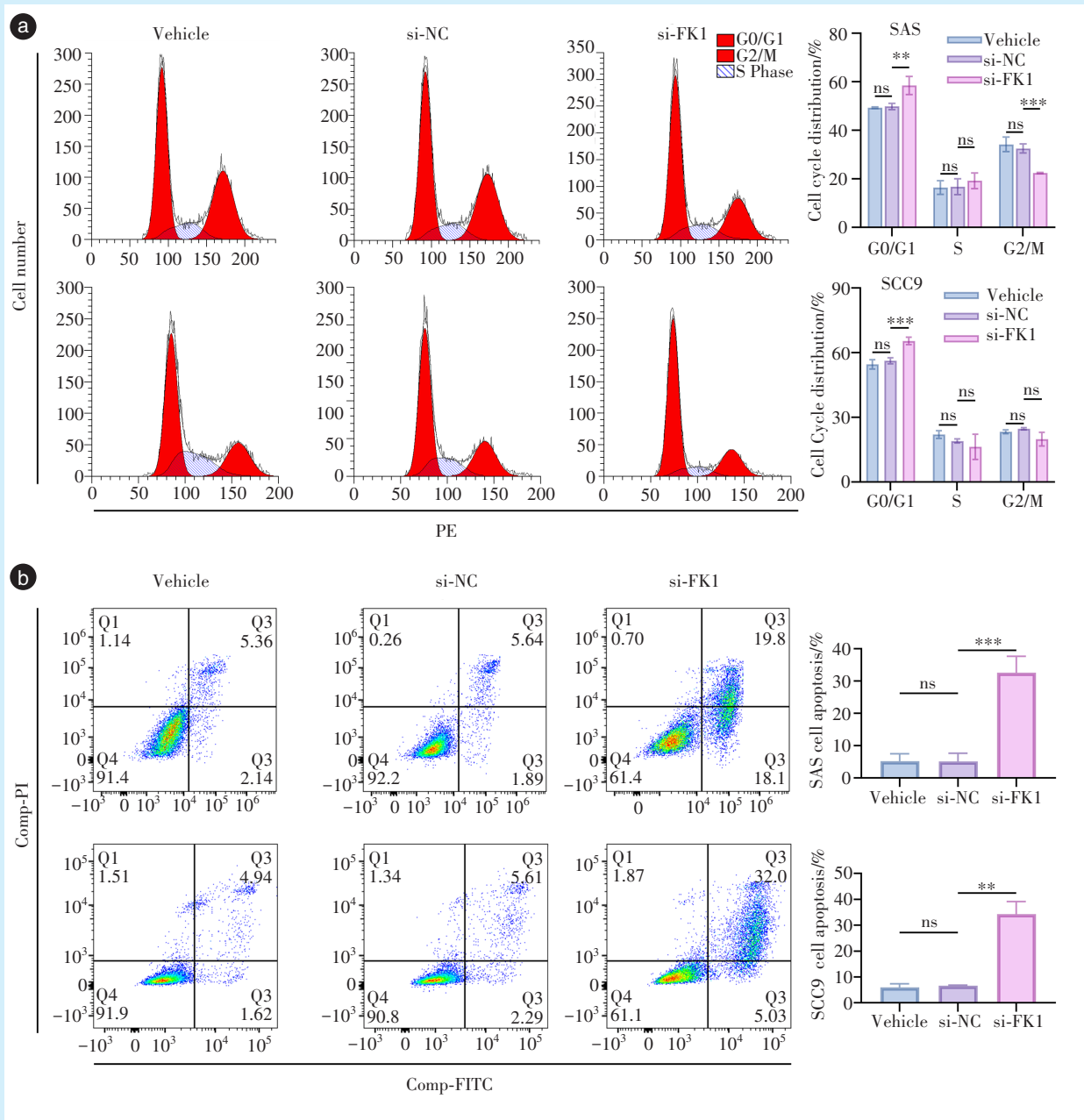
与对照组和 si-NC 组相比, FKBP10 沉默显著减少了 SAS 和 SCC9 细胞从两侧向伤口的迁移 ($P < 0.001$, 图 4)。在侵袭测定中(图 5), FKBP10 沉默同样显著降低了 SAS 和 SCC9 细胞的侵袭 ($P < 0.001$)。因此, FKBP10 通过改变肿瘤细胞的迁移和侵袭行为来影响癌症的转移和进展。

2.3 FKBP10 通过降低 RAP1-PI3K-AKT 信号通路转导影响 OSCC 进展

蛋白质液相色谱质谱蛋白差异表达分析的结果显示(图 6a), 在 FKBP10 沉默的基础上, 有 217 个蛋白的表达差异有统计学意义, 其中表达上调的蛋白有 107 个, 表达下调的蛋白有 110 个。如表 3 和表 4 所示, 列举了 FKBP10 敲低后 SAS 和 SCC9 细胞中 10 种显著上调/下调蛋白变化。在 Ras 相关蛋

白 1 (Ras-related protein 1, RAP1) 信号通路中, RAP 鸟嘌呤核苷酸交换因子 1 (rap guanine nucleotide exchange factor 1, RAPGEF1) 显示出最为强烈的表达下调, 而 FERM、ARH/RhoGEF 和 pleckstrin 域蛋白 2 (FERM, ARH/RhoGEF and pleckstrin domain protein 2, FARP2) 和腺苷酸环化酶 7 (adenylate cyclase 7, ADCY7) 的表达水平也产生了一定程度的下降。

将具有统计学差异的蛋白进行京都基因与基因组百科全书 (Kyoto encyclopedia of genes and genome, KEGG) 信号通路富集分析, 结果显示(图 6b) 致病性大肠杆菌感染、脂肪的消化和吸收、凋亡通路等在上调的通路中存在显著上升。而在 FKBP10 表达沉默后的信号通路下调分析中(图 6c), RAP1 信号通路、人巨细胞病毒感染、神经生



a: in the cell cycle experiment, SAS and SCC9 in the si-FK1 group were blocked in the G0/G1 phase; b: in the apoptosis experiment, SAS and SCC9 apoptosis rates in the si-FK1 group were promoted. Vehicle group: only the transfection reagent group; si-NC group: negative control group; si-FK1 group: si-FKBP10 sequence 1 group; FKBP10: FK506 binding protein 10; OSCC: oral squamous cell carcinoma. $n=3$, ns: not significant, ** $P < 0.01$, *** $P < 0.001$

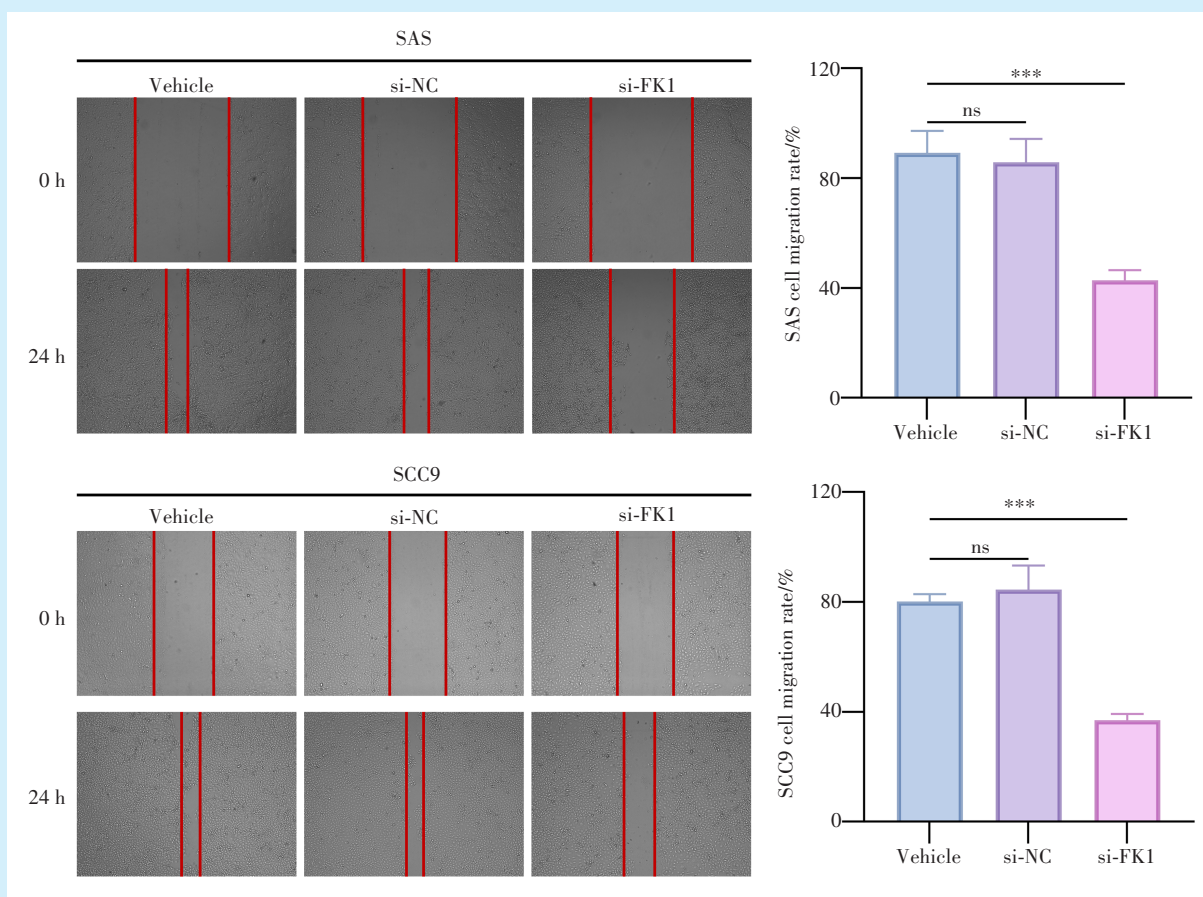
Figure 3 The effect of FKBP10 knockdown in human OSCC cell line SAS and SCC9 on cell cycle and apoptosis

图3 FKBP10在人OSCC细胞系SAS和SCC9细胞中敲低后对细胞周期和凋亡的影响

长因子信号通路产生显著下降,其中RAP1信号通路的变化差异最为显著,呈现出最强的相关性。因此,本研究认为FKBP10对RAP1信号通路的调节是控制OSCC进展的重要机制。

进一步探讨FKBP10敲低对磷脂酰肌醇3激酶(phosphatidylinositol 3-kinase, PI3K)和丝氨酸/苏氨

酸激酶(protein kinase B, AKT)磷酸化水平的改变。PI3K和AKT经过磷酸化后产生功能活性,而通过48 h干扰后,FKBP10的耗竭降低了PI3K($P < 0.05$)和AKT蛋白($P < 0.05$)的磷酸化形式(图7)。表明FKBP10通过改变RAP1信号通路转导而降低PI3K和AKT活化,从而促使OSCC细胞功能产生变化。



in the wound healing experiment, the migration ability of SAS and SCC9 cells was significantly inhibited in the si-FK1 group compared with the Vehicle group and si-NC group. Vehicle group: only the transfection reagent group; si-NC group: negative control group; si-FK1 group: si-FKBP10 sequence 1 group; FKBP10: FK506 binding protein 10; OSCC: oral squamous cell carcinoma. $n=3$, ns: not significant, *** $P < 0.001$

Figure 4 The effect of FKBP10 knockdown in human OSCC cell line SAS and SCC9 on cell migration

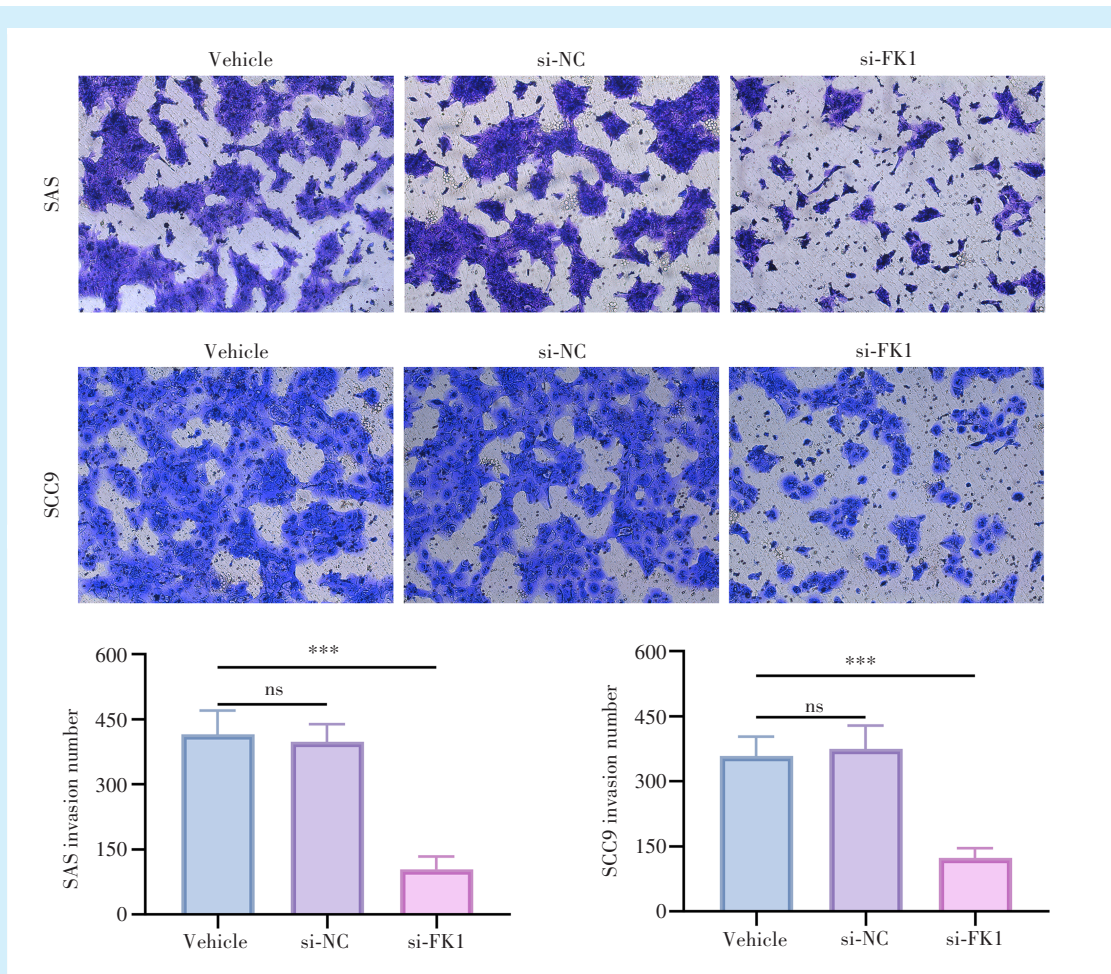
图4 FKBP10在人OSCC细胞系SAS和SCC9细胞中敲低后对细胞迁移的影响

3 讨论

口腔癌是一种能够依靠头面部丰富的血管和淋巴结而快速生长并早期转移的癌症。因此,早期诊断对于提高OSCC患者的生存率至关重要。尽管非线性成像组织病理学优化了术中病理癌症评估^[17],但其术前诊断作用较低。因此,众多患者在确诊OSCC时已然到达癌症中晚期状态,对治疗和术后恢复挑战较大。此外,肿瘤异质对癌症的诊断、治疗和预后也存在极大影响^[18]。因此,基于个体化分子靶点及相关机制的精准治疗成为了科学家研究靶向药物和开发治疗策略的重点^[19-20]。

在本研究中,基于TCGA数据库的OSCC病例分析显示,肿瘤组织的FKBP10表达显著上调。其表达模式与既往研究中细胞内信号转导、翻译调控及代谢重编程驱动肿瘤恶性表型一致^[12-13]。鉴于肿瘤生物学特性与组织病理学分级之间的相关

性,检测FKBP10的丰度有助于OSCC的早期诊断和预后生存期的评估。此外,FKBP10功能沉默导致SAS和SCC9细胞迁移和侵袭能力显著下降,提示抑制FKBP10可能通过改变上皮间充质转化或重塑细胞外基质产生关键作用,类似于其在增生性瘢痕形成中的生物学功能^[21]。值得注意的是,FKBP10沉默阻滞了细胞周期进展并显著增强SAS和SCC9细胞的凋亡。目前,尽管细胞自噬^[22]和铁死亡^[23]被认为是未来癌症临床治疗转化的潜在途径,而以凋亡为主的细胞调节性死亡对于化疗耐药和复发性癌症仍是一种有效的治疗策略^[24]。但与之矛盾的是,肿瘤细胞群中的局限性细胞凋亡也有可能通过改变肿瘤微环境产生耐药性^[25]。因此,与蛋白靶点抑制剂协同凋亡机制类似^[26-27],本研究的发现有助于将FKBP10抑制作为增强促凋亡药物疗效的潜在分子靶点。



in the transwell experiment, the migration ability of SAS and SCC9 cells was significantly inhibited in the si-FK1 group compared with the Vehicle group and si-NC group. Vehicle group: only the transfection reagent group; si-NC group: negative control group; si-FK1 group: si-FKBP10 sequence 1 group; FKBP10: FK506 binding protein 10; OSCC: oral squamous cell carcinoma. $n=3$, ns: not significant, *** $P < 0.001$

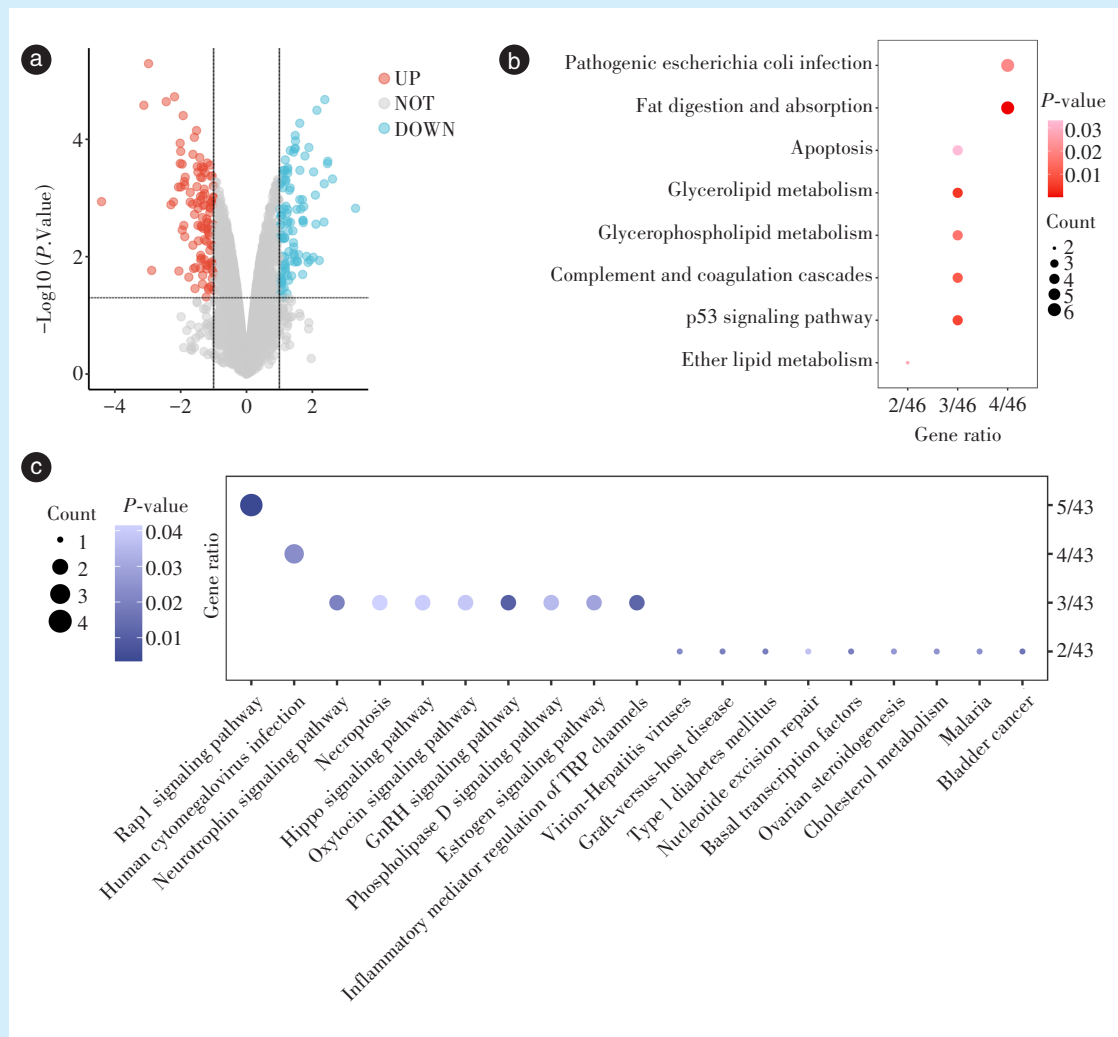
Figure 5 The effect of FKBP10 knockdown in human OSCC cell line SAS and SCC9 on cell invasion

图5 FKBP10在人OSCC细胞系SAS和SCC9细胞中敲低后对细胞侵袭的影响

从机制上讲,蛋白表达差异分析和KEGG信号通路富集分析表明,FKBP10沉默导致RAP1信号通路相关蛋白丰度降低,如RAPGEF1、ADCY7和FARP2。这些RAP1信号通路的相关蛋白质在多种癌症中具有重要作用,如胶质母细胞瘤^[28-29]和肝细胞性肝癌^[30]。RAP1是Ras超家族的一种小G蛋白,其在非活性二磷酸鸟苷(guanosine diphosphate, GDP)结合和活性磷酸鸟苷(guanosine triphosphate, GTP)结合构象之间的循环能够调节细胞功能,如内皮细胞介导的血管通透性和屏障功能^[31-32]。此外,RAP1可与头孢喹啉结合并产生活性,从而参与细胞迁移和侵袭等生物学过程^[33]。更重要的是,RAP1对PI3K-AKT和丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)信号通路的双重调节控制癌症进展。一方面,RAP1激

活可通过抑制MAPK信号轴延缓甲状腺癌进展,或阻断MAPK信号通路以抑制Ras的致癌活性^[34]。另一方面,RAP1对下游PI3K-AKT信号通路的影响也存在串扰,如小檗碱通过RAP1-PI3K-AKT轴改变胰腺癌对吉西他滨的耐药性^[35];其下游分子RAPGEF1与AKT通路中糖原合成酶激酶3 β (glycogen synthase kinase 3, G3K3 β)结合并相互调节^[36],以及间充质干细胞中鸢尾素通过RAP1-PI3K-AKT转导调节软骨分化^[37]。更重要的是,本研究表明FKBP10沉默通过RAP1-PI3K-AKT轴抑制OSCC癌症进展。因此,这些发现均提示靶向分子靶点的疾病精准治疗策略是未来主要的研究方向。

FKBP10的临床意义不仅存在于对肿瘤细胞生物学行为的调控作用,其对促胶原折叠、肿瘤微环



a: analysis of protein differential expression after FKBP10 knockdown in the si-FK1 group compared to the si-NC group; b & c: compared with the Vehicle group, KEGG pathway analysis of upregulated (b) and downregulated (c) expression in the FKBP10 knockdown group. Vehicle group: only the transfection reagent group; si-NC group: negative control group; si-FK1 group: FKBP10 sequence 1 group; FKBP10: FK506 binding protein 10; OSCC: oral squamous cell carcinoma; KEGG: Kyoto Encyclopedia of Genes and Genomes

Figure 6 Proteomic analysis of FKBP10 knockdown in human OSCC cell line SAS and SCC9 cells

图6 人OSCC细胞系SAS和SCC9细胞中FKBP10敲低后的蛋白质组学分析

表3 FKBP10敲低后人OSCC细胞系SAS和SCC9细胞中10种显著上调蛋白变化

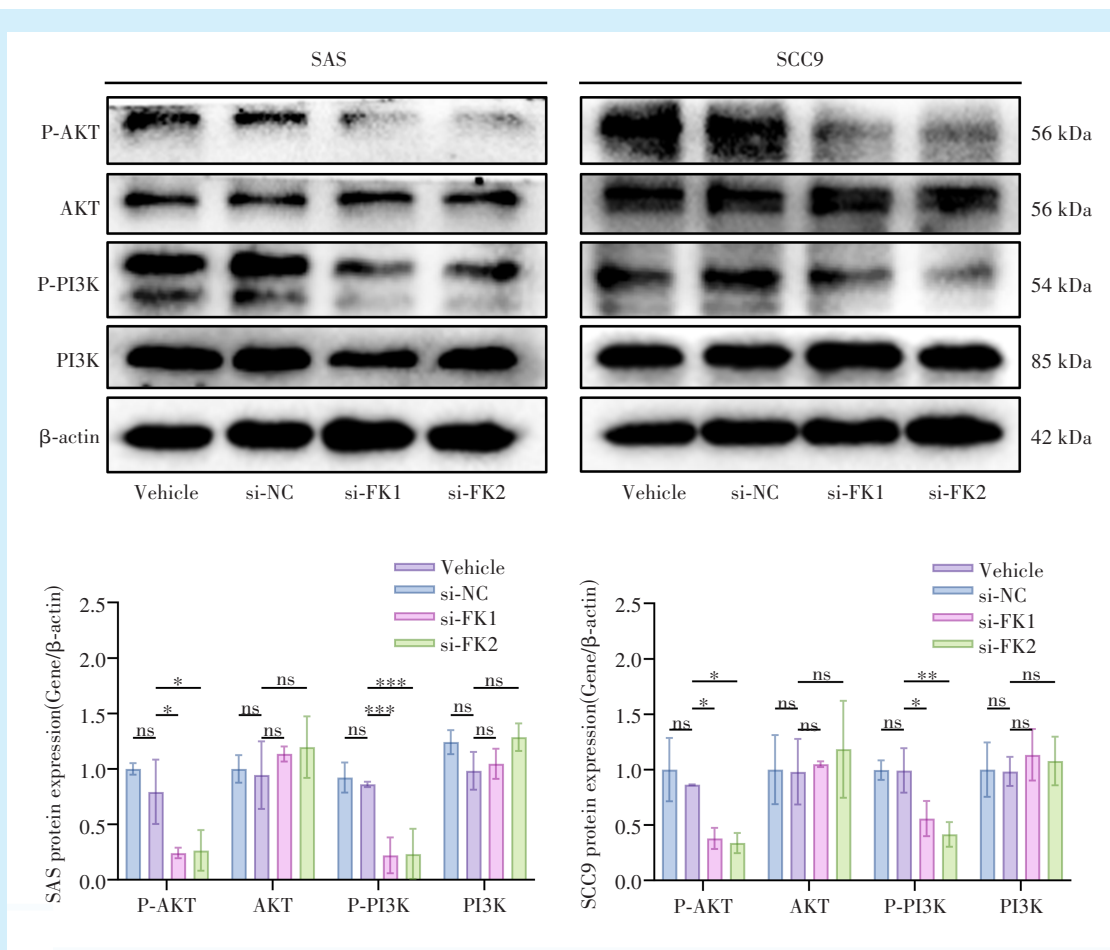
Table 3 Ten significantly upregulated protein changes in human OSCC cell line SAS and SCC9 cells after FKBP10 knockdown

Sequence number	English abbreviation	Full name in English
1	DPP8	Dipeptidyl peptidase 8
2	SPRR2A	Small proline-rich protein 2A
3	PLPP2	Phospholipid phosphatase 2
4	PCBP4	Poly(rC)-binding protein 4
5	KAT6A	Histone acetyltransferase KAT6A
6	B3GNT3	N-acetylglucosaminide beta-1, 3-N-acetylglucosaminyltransferase 3
7	SPRR2F	Small proline-rich protein 2F
8	TAF6	Transcription initiation factor TFIID subunit 6
9	PNPLA2	Patatin-like phospholipase domain-containing protein 2
10	MYADM	Myeloid-associated differentiation marker

表4 FKBP10敲低后人OSCC细胞系SAS和SCC9细胞中10种显著下调蛋白变化

Table 4 Ten significantly downregulated protein changes in human OSCC cell line SAS and SCC9 cells after FKBP10 knockdown

Sequence number	English abbreviation	Full name in English
1	RAPGEF1	Rap guanine nucleotide exchange factor 1
2	ALKBH6	Alpha-ketoglutarate-dependent dioxygenase alkB homolog 6
3	FGFBP1	Fibroblast growth factor-binding protein 1
4	TXNDC11	Thioredoxin domain-containing protein 11
5	HELLS	Lymphoid-specific helicase
6	CD109	CD109 antigen
7	FARP2	FERM, ARHGEF and pleckstrin domain-containing protein 2
8	LTBP2	Latent-transforming growth factor beta-binding protein 2
9	ZMYM3	Zinc finger MYM-type protein 3
10	ADCY7	Adenylate cyclase type 7



Assessment of P-AKT, AKT, P-PI3K, and PI3K protein expression following transfection of si-NC, si-FK1, and si-FK2 in SAS and SCC9 cells. Vehicle group: only the transfection reagent group; si-NC group: negative control group; si-FK1 and si-FK2 groups: knockdown group using sequence 1 or 2; P-AKT: phosphorylated protein kinase B, AKT: protein kinase B, P-PI3K: phosphorylated phosphatidylinositol 3-kinase, PI3K: phosphatidylinositol 3-kinase. $n=3$. ns: not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

Figure 7 Changes in phosphorylation levels of PI3K-AKT signaling pathway in human OSCC cell line SAS and SCC9 cells

图7 人OSCC细胞系SAS和SCC9细胞中PI3K-AKT信号通路磷酸化水平变化

境免疫调节和细胞外基质生态位也具有重要作用^[38-39]。未来的研究应注重FKBP10对细胞外基质

重塑的贡献并评估其对一线治疗的预测价值,从而验证其预后生物标志物和治疗靶点的双重效

用。本研究虽初步揭示了FKBP10在OSCC中的生物学功能及其作用机制,但仍存在局限性。首先,在临床验证层面,研究尚未通过临床病例进一步确认FKBP10在口腔癌患者组织中的差异表达特征;其次,在机制研究方面,虽然已证实FKBP10通过调控RAP1-PI3K-AKT信号轴影响了肿瘤恶性进展,但未能深入探讨FKBP10基因沉默对RAP1另外一条下游MAPK信号通路级联反应的潜在调控作用,这在一定程度上限制了对RAP1在口腔癌中分子调控机制的全面解析。后续将优化实验设计方法,收集数据并着重探究FKBP10对RAP1信号通路中特定蛋白质受体及下游信号通路的改变,且在动物水平验证结果的可靠性。

综上所述,本研究通过FKBP10介导的增殖、侵袭和凋亡的调控,确定了FKBP10是OSCC发病机制中的一种多功能癌蛋白。此外,细胞模型中FKBP10耗竭对恶性表型的抑制强调了其治疗潜力。本研究为临床口腔肿瘤治疗中开发FKBP10靶向诊断策略、预后评估以及精准治疗提供了依据。

【Author contributions】 Fang ZK designed the study, performed the experiments, and wrote the article. Jin H, Yang S, Jiang N and Zhang MY designed the research study and revised the article. Zhou S, Li C analyzed the data and revised the article. Li LL conceptualized and reviewed the article. All authors read and approved the final manuscript as submitted.

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