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ITGA6通过PI3K/AKT信号通路调控腹壁子宫内膜异位症的机制研究

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摘要 目的 探讨整合素α6(ITGA6)在腹壁子宫内膜异位症(AWE)组织中的表达情况及其参与调控AWE的分子机制。方法 收集腹壁子宫内膜异位组织36例作为实验组,正常在位子宫内膜组织36例作为对照组,免疫组织化学技术评估ITGA6在两种组织中的表达差异;设计、合成人ITGA6基因特异性干扰序列,慢病毒包装后转染Ishikawa细胞(人子宫内膜腺癌细胞),构建ITGA6低表达细胞株;根据其基因编码序列(CDS)构建ITGA6过表达细胞株;Real-time PCR及Western blot检测上皮-间质转化(EMT)及血管生成相关指标的变化;细胞划痕、Transwell实验检测细胞侵袭迁移能力;Western blot检测磷脂酰肌醇-3激酶/蛋白激酶B(PI3K/AKT)信号通路的变化。结果 与在位子宫内膜相比,腹壁子宫异位内膜组织中ITGA6阳性细胞数及表达强度均明显增强($P < 0.001$);与NC组相比,ITGA6低表达组N-cadherin、VEGF、TGF-β1表达均降低(均 $P < 0.01$),E-cadherin表达明显升高($P < 0.01$),细胞侵袭迁移能力明显降低($P < 0.001$),AKT磷酸化水平显著降低($P < 0.001$)。过表达ITGA6则与上述相反。结论 ITGA6通过激活PI3K/AKT信号通路促进Ishikawa细胞EMT、血管生成和侵袭迁移能力,参与AWE的发生发展。

关键词 整合素α6;子宫内膜异位症;上皮-间质转化;血管生成;细胞黏附

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腹壁子宫内膜异位症(abdominal wall endometriosis, AWE)是子宫内膜异位症(endometriosis, EMS)的一种特殊类型,根据全球流行病学调查显示,发病率约占EMS的0.03%至0.45%^[1],其特点是子宫内膜组织种植于腹壁,伴有浸润性生长^[2],常继发于妇产科手术,剖宫产是最大的危险因素。前期研究^[3]已经证实EMS发病机制与肿瘤类似,病理过程涉及细胞黏附、基质侵袭性破坏和新生血管生成等关键步骤。作为跨膜黏附受体,整合素家族已被证实在肿瘤中参与多种功能调控,整合素α6(integrin alpha-6, ITGA6)发挥的作用尤为突出^[4]。Rai et al^[5]通过免疫组化定量分析显示,内异症患者的在位内膜中,ITGA6表达量与盆腔的异位内膜差异有统计学意义,提示其可能参与疾病进程。但在

AWE中的功能调控未被研究。因此,该研究探讨了ITGA6与AWE之间的关系,通过相关实验揭示ITGA6在AWE发生和进展中的作用机制。

1 材料与方法

1.1 病例资料 将安徽医科大学第一附属医院妇产科2018年1月至2023年10月收治的36例AWE患者纳入研究,均行开放性腹壁内异症切除术,年龄32~43(36.05±3.18)岁,I型(皮下脂肪型)26例,II型(腹直肌型)7例,III型(腹膜型)3例。另选同时期良性肿瘤行子宫切除术36例作为对照组,年龄31~42(37.36±2.97)岁。运用SPSS 27统计软件分析,两组患者年龄差异无统计学意义($P>0.05$)。入选标准:月经规律,组织经病理学证实;排除标准:妊娠,内分泌及代谢性疾病,恶性肿瘤,术前6个月使用激素治疗。

1.2 材料 人子宫内膜癌细胞(Ishikawa)及专用培养基购自武汉普诺赛生命科技有限公司,货号:CL-0823和CM-0283;ITGA6干扰及过表达慢病毒购自上海吉凯基因科技有限公司,货号:

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GIEE0441941 和 GCPE5002046; RNA 提取试剂盒购自信天翁生物科技有限公司(广州);反转录试剂盒、SYBR Green Pro Taq HS 预混型 qPCR 试剂盒购自艾科瑞生物有限公司(湖南);RIPA 细胞裂解液(强)、BCA 蛋白定量试剂盒、SDS-PAGE 凝胶制备试剂盒、彩虹预染蛋白 Marker 购自上海雅酶生物医药科技有限公司;ECL 超敏化学发光检测试剂盒购自碧云天生物技术有限公司(上海)。ITGA6 抗体购自爱博泰克(武汉)生物科技有限公司,货号:A3236;GAPDH 抗体购自武汉贝茵莱生物科技有限公司,货号:MAB45855;转化生长因子-β1(transforming growth factor-Beta1, TGF-β1)抗体(货号:AF1027),血管内皮生长因子(vascular endothelial growth factor, VEGF)抗体(货号:AF5131),神经钙黏蛋白(N-cadherin)抗体(货号:AF6710),上皮钙黏蛋白(E-cadherin)抗体(货号:AF0131)均购自江苏亲科生物研究有限公司。

1.3 方法

1.3.1 免疫组织化学 石蜡切片二甲苯脱蜡,依次经过乙醇浓度梯度实现水化,采用(pH6.0)柠檬酸钠于96℃条件下实现抗原修复,自然降温后PBS冲洗3次,3% H₂O₂孵育15 min消除内源性过氧化物酶活性。依次滴加1:1 000稀释的ITGA6一抗4℃孵育过夜。HRP标记二抗(室温下30 min),DAB显色、苏木精复染,切片制好后显微镜下分析结果。

1.3.2 细胞培养 专用培养基培养Ishikawa细胞(培养箱条件:37℃、5%CO₂),隔天换液,倒置显微镜下观察其生长,待其汇合度为70%~80%时无菌PBS清洗,0.25%胰蛋白酶消化3~5 min,细胞计数后,遵循实验设计进行种板及检测。

1.3.3 ITGA6 低表达及过表达细胞株构建 针对CDS序列设计3条特异性干扰序列并将其克隆到慢病毒载体GV493上,分别标记为siRNA-I(TTC TCCATCGAATATGTGCTC)、siRNA-II(ATTATTC CATCTGCCCTTGCTG)和siRNA-III(GTTTCCAGCA ATTGAATATCC)。同时,设置阴性对照组,标记为NC(TTCTCCGAACGTGTACGT)。根据人基因库中ITGA6(NM_000210.4)CDS序列,将其克隆至慢病毒过表达质粒GV492上,基因测序鉴定后进行细胞转染。依照慢病毒试剂盒说明书成功感染Ishikawa细胞,3 d后按比例传代(1:5)。嘌呤霉素筛选ITGA6低表达及过表达细胞株,其浓度1.0 μg/mL。

1.3.4 Real-time PCR 提取细胞总RNA,Nano300

定量。反转录后应用Real-time PCR检测各目的基因mRNA表达。反应体系配置:2×SYBR Green mixture, 10 μL; 10 μmol/L Forward primer, 0.4 μL; 10 μmol/L Reverse primer, 0.4 μL;cDNA模板, 2.0 μL;ddH₂O, 7.2 μL;反应程序:预变性95℃,30 s;变性95℃,5 s;退火及延伸60℃,30 s。以GAPDH作为内参,采用2^{-ΔΔCt}法分析各基因表达量。见表1。

表1 Real-time PCR引物序列
Tab. 1 The sequence of real-time PCR primers

Gene name	Sequence (5'-3')	Product size (bp)
<i>GAPDH</i>	F: TCGGAGTCAACGGATTTGGTC	96
	R: TGAAGGGTCAATTGATGGCA	
<i>ITGA6</i>	F: GCAGCCTCAACTTGGACAC	129
	R: CACGAGCAACAGCCGCTT	
<i>VEGF</i>	F: CAACAAATGTGAATGCAGACCAA	157
	R: GCTCCAGGGCATTAGACAGC	
<i>TGF-β1</i>	F: GTACCTGAACCCGTGTTGCT	217
	R: CCTCAATTCCCCCTCACCG	
<i>N-cadherin</i>	F: CCTTTCTGCCATCAGCTTGT	74
	R: CTCCTGGTTCCAGCTTGT	
<i>E-cadherin</i>	F: GCTGGACCGAGAGAGTTCC	179
	R: CGACGTTAGCCTCGTTCTCA	

1.3.5 Western blot 提取细胞总蛋白,BCA定量,SDS-PAGE分离目的蛋白。经转膜、封闭和一、二抗孵育后,采用全自动化学发光检测仪进行ECL法检测目的蛋白。Quantity One软件进行灰度,计算各自的蛋白/GAPDH。

1.3.6 细胞划痕实验 使用Marker标记笔在6孔细胞培养板背面进行划痕标记,待细胞生长至汇合度60%~70%时,使用直径为1 mm的划痕器垂直于培养板、沿标记线进行细胞划痕标记,无菌PBS洗涤细胞3次,加入2 mL细胞培养基,显微镜下拍照,记为0 h。同样,分别于24 h和48 h拍照留取对应点的细胞划痕图片。以NC组作为对照,计算细胞迁移率,并进行统计分析。细胞迁移率=(细胞划痕宽度_{0 h}-细胞划痕宽度_{24 h/48 h})/细胞划痕宽度_{0 h}×100%。

1.3.7 Transwell实验 细胞消化、计数,于上室中加入200 μL无血清细胞悬液,下室为含10% FBS的细胞培养液。37℃,5%CO₂细胞培养箱继续培养24 h。取出小室,PBS洗涤3次,4%多聚甲醛固定30 min。PBS洗涤3次,苏木紫染色15 min,用棉签轻柔拭去上层非迁移细胞,PBS洗涤3次。随机选取5个视野细胞进行拍照,计数后进行统计分析。

1.4 统计学处理 采用GraphPad Prism5.0软件进行统计分析,数据均用 $\bar{x}\pm s$ 表示。采用t检验验证两组之间差异性,采用单因素方差分析检验多组间的差异性, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 免疫组织化学分析 图1显示,在位子宫内膜腺体结构完整、形态规则;异位子宫内膜腺体结构严重破坏、纤维增生明显、细胞形态不规则。ITGA6主要表达于腺上皮细胞和间质细胞细胞膜上,与在位内膜组织相比,腹壁异位内膜组织中ITGA6的表达水平明显升高,阳性细胞数显著增多($t=7.064, P < 0.001$)。

2.2 低表达和过表达ITGA6细胞株构建 与GV493/NC组相比,siRNA-I组和siRNA-II组ITGA6 mRNA和蛋白表达均显著降低($t=6.445, P < 0.001; t=8.978, P < 0.001$),siRNA-III组无明显变化,差异无统计学意义;与siRNA-I组相比,siRNA-II组ITGA6 mRNA和蛋白降低($t=2.432, P < 0.05; t=2.949, P < 0.05$),干扰效率更高。见图2。

选用siRNA-II序列构建ITGA6低表达细胞株(GV493/siRNA)。与GV493/NC组相比,GV493/siRNA组ITGA6 mRNA和蛋白均降低,差异有统计学意义($t=7.341, P < 0.001; t=8.512, P < 0.001$),证实ITGA6低表达细胞株构建成功。将慢病毒包

装的ITGA6过表达载体转染至Ishikawa细胞,与GV492/NC组相比,ITGA6过表达细胞株(GV492/ITGA6)ITGA6 mRNA和蛋白表达均明显升高($t=8.526, P < 0.001; t=4.753, P < 0.001$),差异有统计学意义,证实ITGA6过表达细胞株构建成功。见图3。

2.3 ITGA6对EMT及血管生成相关指标的影响 Real-time PCR和Western blot检测EMT和血管生成相关指标上E-cadherin、N-cadherin、VEGF、TGF- β 1 mRNA和蛋白的表达。结果显示,与GV493/NC组相比, GV493/siRNA组 N-cadherin、VEGF 和 TGF- β 1 表达明显降低($t=4.651, P < 0.001; t=5.153, P < 0.001; t=4.446, P < 0.01$),蛋白表达也明显降低($t=9.097, P < 0.001; t=3.188, P < 0.01; t=6.905, P < 0.001$);E-cadherin表达明显升高($t=3.272, P < 0.001$)。见图4。

同样,与GV492/NC组相比, GV492/ITGA6组 N-cadherin、VEGF 和 TGF- β 1 mRNA 表达则显著升高($t=7.158, P < 0.001; t=13.78, P < 0.001; t=6.732, P < 0.001$),蛋白表达也显著升高($t=4.431, P < 0.01; t=6.393, P < 0.001; t=11.400, P < 0.001$);E-cadherin mRNA 表达明显降低($t=6.992, P < 0.001$),蛋白表达也明显降低($t=10.990, P < 0.001$)。见图5。

2.4 ITGA6对细胞侵袭迁移能力的影响

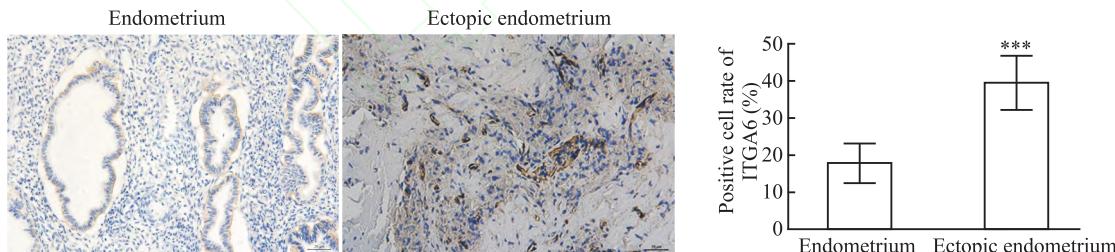


图1 正常内膜与异位内膜ITGA6免疫组化分析 $\times 20$

Fig. 1 Comparative IHC staining of ITGA6 in ectopic endometrium and endometrium $\times 20$

*** $P < 0.001$ vs Endometrium.

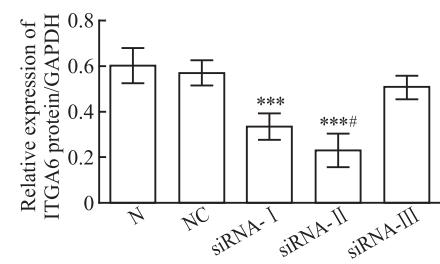
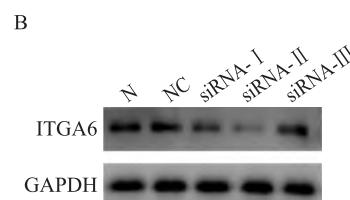
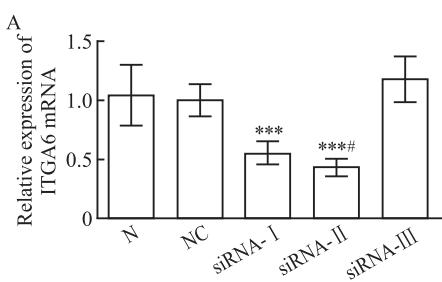


图2 ITGA6干扰序列筛选

Fig. 2 Screening of effective siRNA constructs for ITGA6 knockdown

A: The mRNA expression level of siRNA-I, II, III; B: The Western blot result of siRNA-I, II, III; *** $P < 0.001$ vs NC group; # $P < 0.05$ vs siRNA-I.

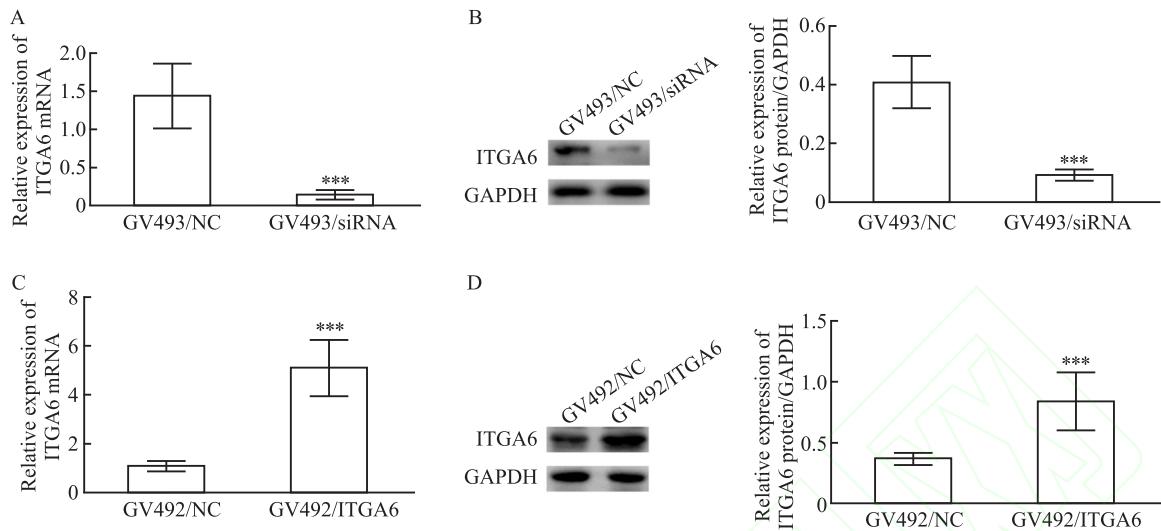


图3 ITGA6低表达及过表达细胞株构建

Fig. 3 Construction of ITGA6 knockdown and overexpression cell lines

A: The mRNA expression level of ITGA6 knockdown group; B: The Western blot result of ITGA6 knockdown group; C: The mRNA expression level of ITGA6 overexpression group; D: The Western blot result of ITGA6 overexpression group; *** $P < 0.001$ vs NC group.

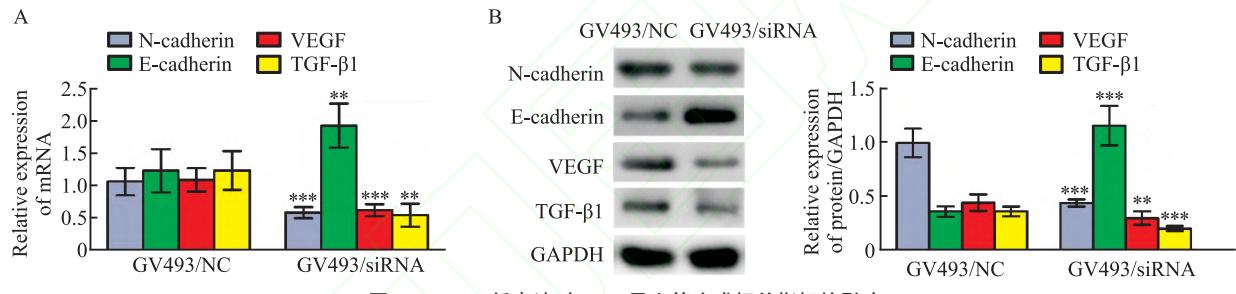


图4 ITGA6低表达对EMT及血管生成相关指标的影响

Fig. 4 The effects of ITGA6 knockdown on EMT and angiogenesis

A: The mRNA expression level of E-cadherin, N-cadherin, VEGF and TGF-β1 in ITGA6 knockdown group; B: The Western blot result of E-cadherin, N-cadherin, VEGF and TGF-β1 in ITGA6 knockdown group; ** $P < 0.01$, *** $P < 0.001$ vs NC group.

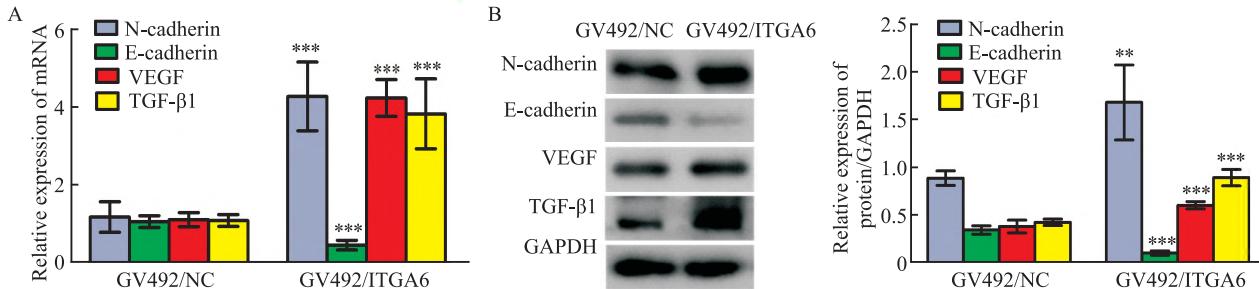


图5 ITGA6过表达对EMT及血管生成相关指标的影响

Fig. 5 The effects of ITGA6 overexpression on EMT and angiogenesis

A: The mRNA expression level of E-cadherin, N-cadherin, VEGF and TGF-β1 in the ITGA6 overexpression group; B: The Western blot result of E-cadherin, N-cadherin, VEGF and TGF-β1 in the ITGA6 overexpression group; ** $P < 0.01$, *** $P < 0.001$ vs NC group.

2.4.1 ITGA6低表达对细胞侵袭迁移能力的影响 Transwell检测及分析结果表明,与GV493/NC组相比, GV493/siRNA组细胞穿透数量显著减少, 细胞侵袭能力明显降低($t=8.267, P < 0.001$)。见图6。

细胞划痕检测及分析结果表明,与GV493/NC组相比, GV493/siRNA组24、48 h的细胞划痕宽度显著增加, 细胞迁移能力明显降低($t=5.265, P < 0.001$; $t=8.267, P < 0.001$)。见图7。

2.4.2 ITGA6过表达对细胞侵袭迁移能力的影响

响 Transwell 检测结果表明,与 GV492/NC 组相比, GV492/ITGA6 组细胞穿透数量显著增加,细胞侵袭能力明显升高($t=6.566, P < 0.001$)。见图 8。

细胞划痕检测及结果表明,与 GV492/NC 组相

比, GV492/ITGA6 组细胞 24 h 和 48 h 的细胞划痕宽度均显著减少,细胞迁移能力明显增强($t=3.031, P < 0.05; t=3.656, P < 0.001$)。见图 9。

2.5 ITGA6 对 PI3K/AKT 信号通路的影响

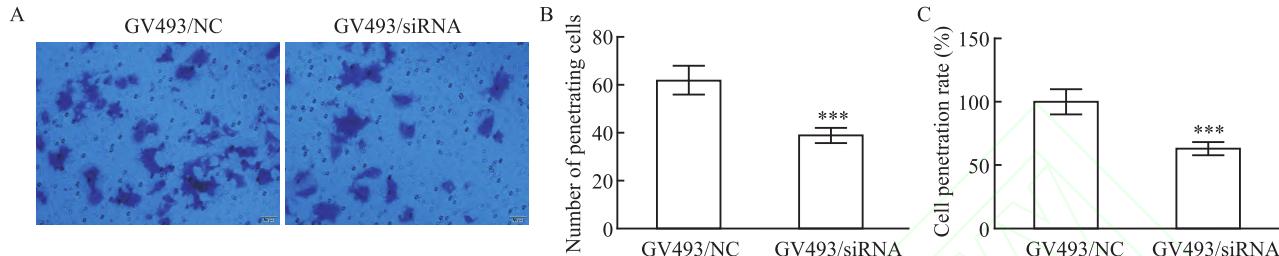


图 6 ITGA6 低表达对细胞侵袭能力的影响

Fig. 6 The effects of ITGA6 knockdown on cell invasion capability

A: Transwell results of GV493/NC and GV493/siRNA groups $\times 20$; B: The results of the cell penetration number in the ITGA6 knockdown group; C: The results of the cell penetration ability in the ITGA6 knockdown group; *** $P < 0.001$ vs NC group.

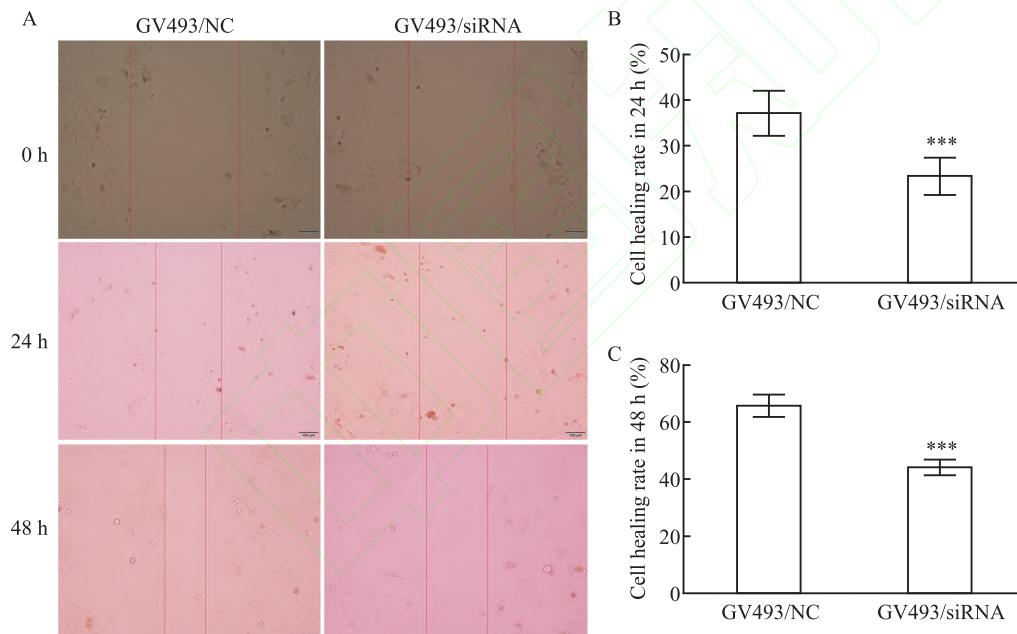


图 7 ITGA6 低表达对细胞迁移能力的影响

Fig. 7 The effects of ITGA6 knockdown on cell migration ability

A: Microscopic images of the ITGA6 knockdown group at 0 h, 24 h and 48 h in the wound healing assay $\times 10$; B: The wound healing rate of the NC group and the ITGA6 knockdown group at 24 h; C: The wound healing rate results of the NC group and the ITGA6 knockdown group at 48 h; *** $P < 0.001$ vs NC group.

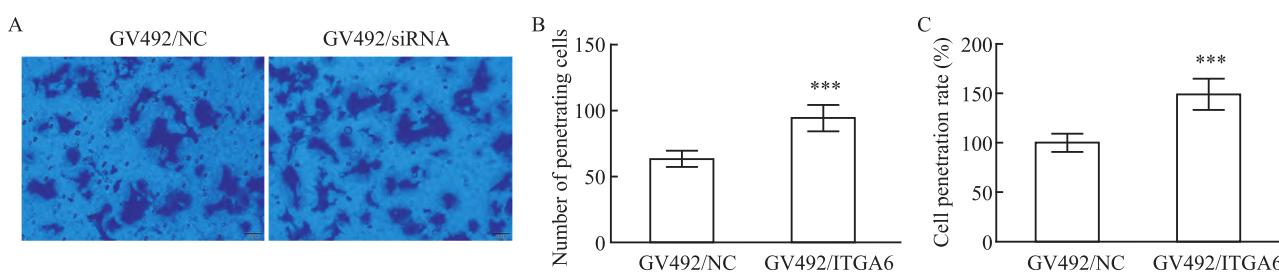


图 8 ITGA6 过表达对细胞侵袭能力的影响

Fig. 8 The effects of ITGA6 overexpression on cell invasive ability

A: The Transwell results of GV492/NC and GV492/ITGA6 groups $\times 20$; B: The results of the cell penetration number in the NC group and the ITGA6 overexpression group; C: The cell penetration ability in the NC group and the ITGA6 overexpression group; *** $P < 0.001$ vs NC group.

Western blot 检测 ITGA6 低表达及过表达对 AKT 及 AKT 磷酸化水平的影响, 研究结果表明, 低表达 ITGA6 降低 AKT 磷酸化水平, 抑制 AKT 磷酸化 ($t=4.753, P < 0.001$); 过表达 ITGA6 增加 AKT 磷酸化水平, 促进 AKT 磷酸化 ($t=7.445, P < 0.001$)。见图 10。

3 讨论

ITGA6 在 AWE 病灶特异性高表达, 可能与异位子宫内膜组织的黏附力增加和手术创伤有关。首先, 整合素是层粘连蛋白主要受体, 促进细胞之间、细胞-基质之间的粘连, 维持细胞基底膜稳定^[6]。在 EMS 机制研究中^[7], 盆腔异位子宫内膜较在位子宫内膜的 ITGA6 表达下降, 致使细胞-细胞或细胞-基

质黏附力下降, 子宫内膜组织易从宫腔脱落游走于盆腔。相反在腹壁子宫内膜异位组织中, ITAG6 表达升高, 使异位的子宫内膜细胞之间黏附力增强、稳定性增加, 促进了子宫内膜细胞植入。其次, 有研究^[8]证实了瘢痕部位的机械应力, 可以激活 YAP/TAZ 信号通路, 使之与 TEAD 转录因子结合上调 ITGA6。剖宫产手术创伤可能激活瘢痕部位机械传导系统, ITGA6 表达增高, 此观点需要相关实验验证。

实验中上调 ITGA6 表达促进细胞 EMT 和血管生成, 增强细胞侵袭、迁移能力和病灶血管新生, 相反低表达则抑制细胞 EMT 和血管生成, 证明腹壁子宫内膜异位病灶不但具备向周围组织迁移侵袭能力, 而且拥有丰富的血液供应。其中 EMT 在 EMS 发

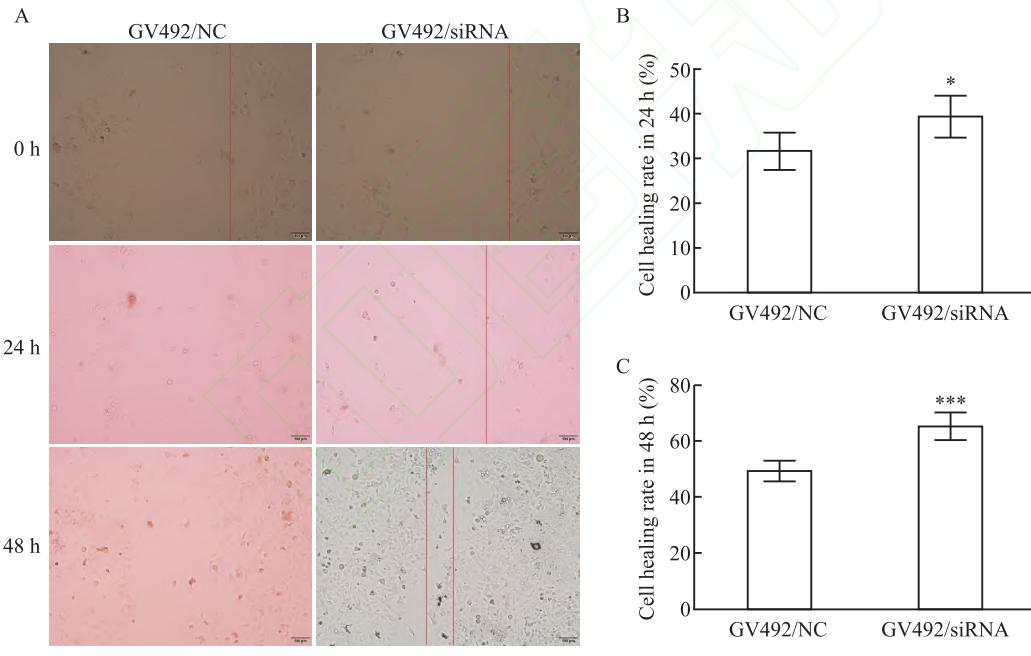


图 9 ITGA6 过表达对细胞迁移能力的影响

Fig. 9 The effects of ITGA6 overexpression on cell migratory ability

A: Microscopic images of the ITGA6 overexpression group at 0 h, 24 h and 48 h in the wound healing assay $\times 10$; B: The wound healing rate results of the NC group and the ITGA6 overexpression group at 24 h; C: The wound healing rate results of the NC group and the ITGA6 overexpression group at 48 h; * $P < 0.05$, *** $P < 0.001$ vs NC group.

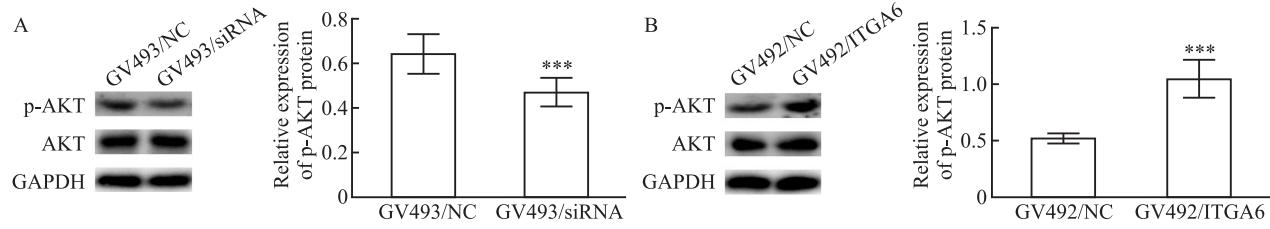


图 10 ITGA6 对 PI3K/AKT 信号通路的影响

Fig. 10 The effects of ITGA6 on the PI3K/AKT signaling pathway

A: The Western blot results of AKT and p-AKT in the ITGA6 knockdown group; B: The Western blot results of AKT and p-AKT in the ITGA6 overexpression group; *** $P < 0.001$ vs NC group.

展中发挥重要作用^[9]。肿瘤细胞极性消失、黏附能力减弱使其具备迁移侵袭特性,上皮细胞必须经过EMT,完成间质细胞转化才能获得此特征^[10]。其分子表型为E-cadherin下调,N-cadherin上调^[11]。另一方面,异位病灶不断经历损伤修复,活化的血小板通过生成TGF-β1,诱导TGF-β/Smad信号通路促进EMT^[12]。RT-PCR及Western blot结果显示,在AWE中N-cadherin和TGF-β1表达升高,提示与其它类型EMS相同,EMT是异位内膜细胞获得侵袭和迁移能力的关键步骤,并且受到上游基因ITGA6调控。

VEGF是调控血管生成的核心因子,通过增加血管通透性,促进血管内皮细胞增殖和新生血管形成。TGF-β1作为多功能调节因子,介导细胞增殖分化,在EMT中发挥协同作用。研究^[13]显示,EMT患者血清中VEGF和TGF-β1水平升高,并与EMS临床分期正相关。在实验中敲低ITGA6表达,VEGF和TGF-β1指标显著下降,证明了腹壁子宫内膜异位病灶需要丰富的血液供应和足够的营养供给才能进行增殖侵袭,同样受到ITGA6调控。ITGA6双重调控血管生成和EMT,抑制ITGA6可以阻断EMT和减少血管生成。运用ITGA6抑制剂,阻断其侵袭和减少血供达到缩小病灶目的,为AWE临床靶向治疗提供新方向。

PI3K/AKT信号转导途径作为细胞内核心调控系统,通过级联磷酸化实现跨膜信号转导,参与调控细胞增殖、程序性死亡以及代谢平衡。它是一个复杂的信号功能轴,受多个上游基因调控,同时又作用于下游靶器官。该通路过度激活促进肿瘤和EMS的发生^[14-15]。细胞因子-受体结合诱发PI3K膜定位并激活,催化磷脂酰肌醇-4,5-二磷酸(PIP2)向磷脂酰肌醇-3,4,5-三磷酸(PIP3)转化,募集AKT使之磷酸化,活化AKT通过磷酸化级联反应调控下游VEGF、mTOR等效应分子。上升和敲低ITGA6表达后,AKT含量随之升落,证明了ITGA6作为上游基因,调控PI3K/AKT信号通路。临床诊疗中,AKT抑制剂通过抑制AKT磷酸化降低细胞恶性生物学行为,也可作为AWE新型靶向治疗^[16]。

AWE病灶中潜在特异性调控机制上调ITGA6表达,激活PI3K/AKT信号传导,诱导下游靶蛋白磷酸化修饰,驱动异位组织细胞增殖和病理血管生成,增加异位细胞侵袭迁移潜能,促进AWE发生发展。本研究从分子层面探索AWE发病机制,为PI3K/AKT靶向治疗奠定理论基础。但仍存在局限

性:①临床样本量较小,免疫组化中未分析ITGA6阳性率是否与AWE临床分型、复发率相关,需要扩大样本量补充结论;②AWE原代细胞获取困难,实验基于Ishikawa细胞系进行研究,远期需要原代细胞或构建动物模型验证实验结果;③虽揭示了AWE中ITGA6升高对PI3K/AKT信号通路的调控,但是缺少对ITGA6上游调控和信号通路下游靶分子作用机制的研究,需要今后更深层次探索。

参考文献

- Mittal R, Ashraf A, Gupta M, et al. Scar endometriosis: the menace of surgery [J]. *J Surg Case Rep*, 2023, 2023 (7) : rjad413. doi:10.1093/jscr/rjad413.
- 支园园, 姜艳艳. 腹壁子宫内膜异位症诊治和预防专家共识(2024年版)[J]. 社区医学杂志, 2025, 23(1) : 1-8. doi: 10.19790/j.cnki.JCM.2025.01.01.
- Zhi Y Y, Jiang Y Y. Expert Consensus on the diagnosis, treatment, and prevention of abdominal wall endometriosis (2024 edition) [J]. *J Community Med*, 2025, 23 (1) : 1-8. doi: 10.19790/j.cnki.JCM.2025.01.01.
- 郎景和. 子宫内膜异位症研究的新里程[J]. 中华妇产科杂志, 2005, 40(1) : 3-4. doi: 10.3760/j.issn:0529-567x.2005.01.002.
- Lang J H. Cornerstone of study on endometriosis [J]. *Chin J Obstet Gynecol*, 2005, 40(1) : 3-4. doi: 10.3760/j.issn:0529-567x.2005.01.002.
- Li S, Sampson C, Liu C, et al. Integrin signaling in cancer: bidirectional mechanisms and therapeutic opportunities [J]. *Cell Commun Signal*, 2023, 21(1) : 266. doi: 10.1186/s12964-023-01264-4.
- Rai V, Hopkisson J, Kennedy S, et al. Integrins alpha 3 and alpha 6 are differentially expressed in endometrium and endometriosis [J]. *J Pathol*, 1996, 180 (2) : 181-7. doi: 10.1002/(SICI)1096-9896(199610)180:2181::AID-PATH620>3.0.CO;2-I.
- Pang X, He X, Qiu Z, et al. Targeting integrin pathways: mechanisms and advances in therapy [J]. *Signal Transduct Target Ther*, 2023, 8: 1. doi:10.1038/s41392-022-01259-6.
- 李亭, 李亚里, 李建民, 等. 整合素α6在子宫内膜异位症中的表达分析[J]. 解放军医学杂志, 2005, 30(5) : 388-90. doi: 10.3321/j.issn:0577-7402.2005.05.009.
- Li T, Li Y L, Li J M, et al. Differential expression of integrin alpha 6 in endometrium and endometriosis [J]. *Med J Chin People's Liberation Army*, 2005, 30(5) : 388-90. doi: 10.3321/j.issn:0577-7402.2005.05.009.
- Dupont S, Morsut L, Aragona M, et al. Role of YAP/TAZ in mechanotransduction [J]. *Nature*, 2011, 474 (7350) : 179-83. doi:10.1038/nature10137.
- Liu L, Liu L, Yue C, et al. PYK2 promotes cell proliferation and epithelial-mesenchymal transition in endometriosis by phosphorylating Snail1 [J]. *Mol Med*, 2025, 31(1) : 155. doi: 10.1186/s10020-025-01218-1.
- Xu S, Zhu C, Xu Q, et al. ARID1A restrains EMT and stemness of ovarian cancer cells through the Hippo pathway [J]. *Int J Oncol*, 2024, 65 (2). DOI: 10.3892/ijo.2024.5664. doi: 10.3892/ijo.2024.5664.

- [11] 胡冰琪, 周 静, 黄俊峰, 等. 双氢青蒿素抑制非小细胞肺癌细胞迁移侵袭和血管生成拟态的初步研究[J]. 安徽医科大学学报, 2023, 58(5): 766-71. doi: 10.19405/j.cnki.issn1000-1492.2023.05.011.
- [11] Hu B Q, Zhou J, Huang J F, et al. A preliminary study of dihydroartemisinin inhibiting migration invasion and vasculogenic mimicry of non-small cell lung cancer cells [J]. Acta Univ Med Anhui, 2023, 58(5): 766-71. doi: 10.19405/j.cnki.issn1000-1492.2023.05.011.
- [12] Zubrzycka A, Migdalska-Sęk M, Jędrzejczyk S, et al. The expression of TGF- β 1, SMAD3, ILK and miRNA-21 in the ectopic and eutopic endometrium of women with endometriosis [J]. Int J Mol Sci, 2023, 24(3): 2453. doi: 10.3390/ijms24032453.
- [13] 刘 佳, 张 燕, 陈少丽. 子宫内膜异位症患者miR-429与HIF-1 α 、VEGF水平和临床分期的相关性及其诊断效能[J]. 广东医学, 2023, 44(11): 1435-9. doi: 10.13820/j.cnki.gdyx.20232183.
- [13] Liu J, Zhang Y, Chen S L. The correlation of miR-429 with HIF-1 α , VEGF levels, and clinical staging in patients with endometriosis and its diagnostic efficacy [J]. Guangdong Med J, 2023, 44(11): 1435-9. doi: 10.13820/j.cnki.gdyx.20232183.
- [14] Li J, Zheng J, Lin B, et al. Knockdown of NCAPG promotes the apoptosis and inhibits the invasion and migration of triple-negative breast cancer MDA-MB-231 cells via regulation of EGFR/JAK/STAT3 signaling [J]. Exp Ther Med, 2023, 25(3): 119. doi: 10.3892/etm.2023.11818.
- [15] Madanes D, Bilotas M A, Bastón J I, et al. PI3K/AKT pathway is altered in the endometriosis patient's endometrium and presents differences according to severity stage [J]. Gynecol Endocrinol, 2020, 36(5): 436-40. doi: 10.1080/09513590.2019.1680627.
- [16] Pervanidis K A, D'Angelo G D, Weisner J, et al. Akt inhibitor advancements: from capivasertib approval to covalent-allosteric promises [J]. J Med Chem, 2024, 67(8): 6052-63. doi: 10.1021/acs.jmedchem.4c00075.

Mechanistic study on ITGA6 regulation of abdominal wall endometriosis via the PI3K/AKT signaling pathway

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Abstract Objective To investigate the differential expression of integrin alpha-6(ITGA6) in abdominal wall endometriosis (AWE) tissues and its molecular mechanisms in regulating AWE. **Methods** 36 AWE lesions were designated as the experimental group, while 36 cases of normal endometrial tissues served as the controls. Differential expression of ITGA6 between the two groups was assessed through immunohistochemical (IHC) staining. Human ITGA6 gene-specific interference sequences were designed, synthesized, and packaged into lentiviral vectors to establish the Ishikawa cell line with ITGA6-knockdown. Similarly, the ITGA6-overexpression cell line was constructed using the coding sequence (CDS) of the gene. Real-time PCR and Western blot were performed to detect changes in epithelial-mesenchymal transition (EMT)-related markers and angiogenesis-related indicators. Cell invasion and migration capabilities were assessed by Cell Scratch and Transwell assays. Furthermore, Western blot was conducted to profile PI3K/AKT pathway dynamics. **Results** Ectopic endometrial tissues exhibited a marked increase in the number of ITGA6-positive cells and their expression intensity compared to eutopic endometrium (each $P < 0.001$). Compared with the NC group, the ITGA6-knockdown group showed significantly reduced expression of N-cadherin, VEGF, and TGF- β 1 ($P < 0.01$), while E-cadherin expression was markedly increased ($P < 0.01$). Concomitantly, the invasion and migration capacities of ITGA6-low expression were significantly impaired ($P < 0.001$ for both), accompanied by a marked reduction in AKT and phosphorylated AKT(p-AKT) levels (all $P < 0.001$). Conversely, overexpressing ITGA6 resulted in opposite effects. **Conclusion** ITGA6 modulates EMT and angiogenesis in Ishikawa cells via the PI3K/AKT signaling pathway, thereby enhancing cell invasion and migration capabilities, which contributes to the pathogenesis of AWE.

Key words integrin alpha-6; endometriosis; epithelial-mesenchymal transition; angiogenesis; cell adhesion

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