

减味寿胎丸通过抑制 Exo-miR-410-3p 摄取促进滋养细胞上皮间质转化、迁移及侵袭减少胚胎流产

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摘要:目的 探讨减味寿胎丸(JSP)对自然流产(SA)的影响,并探究其是否通过抑制滋养细胞摄取外泌体 miR-410-3p(Exo-miR-410-3p)并促进上皮间充质转化(EMT)、迁移及侵袭发挥作用。方法 通过电穿孔方法将 miR-410-3p 模拟物装载入牛奶外泌体以制备 Exo-miR-410-3p,通过透射电子显微镜、纳米颗粒追踪分析和 Western blotting 等方法检测 Exo-miR-410-3p 的表征。制备对照血清或 JSP 含药血清。分别设置对照组:HTR-8/Svneo 正常培养;Exo-miR-410-3p 组:HTR-8/Svneo+Exo-miR-410-3p;Exo-miR-410-3p+对照血清组:HTR-8/Svneo+Exo-miR-410-3p+对照血清;Exo-miR-410-3p+JSP 含药血清组:HTR-8/Svneo+Exo-miR-410-3p+JSP 含药血清。通过 RT-qPCR、Western blotting 和免疫荧光染色检测相关表达差异。分别通过 CCK-8 实验、划痕实验和 Transwell 实验评估 HTR-8/Svneo 细胞增殖、迁移及侵袭能力。对荧光标记外泌体进行体内和体外成像追踪,并计算孕鼠胚胎吸收率。通过 RT-qPCR、Western blotting、原位杂交和免疫组化行表达差异的体内验证。结果 划痕实验和 Transwell 实验显示,与对照组相比,Exo-miR-410-3p 组 HTR-8/Svneo 细胞迁移和侵袭能力减弱($P<0.01$),而 JSP 含药血清可逆转 HTR-8/Svneo 细胞减弱的迁移和侵袭能力。体内实验证实,JSP 含药血清可抑制 Exo-miR-410-3p 到达母胎界面,与 Exo-miR-410-3p 组相比,Exo-miR-410-3p+JSP 组胚胎吸收率更低($P<0.01$)。JSP 含药血清降低 SA 小鼠体内 miR-410-3p 的水平($P<0.05$),同时上调 TRAF6 的表达。结论 JSP 可能通过抑制滋养层细胞摄取 Exo-miR-410-3p,促进 SA 中滋养层细胞 EMT、迁移及侵袭,减少胚胎流产。

关键词:减味寿胎丸;自然流产;外泌体 miR-410-3p;上皮-间质转化;p38 MAPK 信号通路

Jianwei Shoutai Pill reduces spontaneous abortion in mice by inhibiting Exo-miR-410-3p uptake and promoting trophoblast epithelial-mesenchymal transition, migration, and invasion

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Abstract: Objective To investigate the therapeutic effect of Jianwei Shoutai Pill (JSP) on spontaneous abortion (SA) in mice and explore its therapeutic mechanism. **Methods** The miR-410-3p mimic was electroporated into milk exosomes to prepare exosomal miR-410-3p (Exo-miR-410-3p), which was characterized using transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and Western blotting. CCK-8, scratch wound healing, and Transwell assays were used to test the effects of control and JSP-mediated rat serum on proliferation, migration, and invasion of trophoblast HTR-8/Svneo cells. Female C57BL/6 mice were divided into SA model group, Model+Exo-miR-410-3p group, Model+Exo-miR-410-3p+P group, and Model+Exo-miR-410-3p+JSP group. The fluorescently labeled exosomes was tracked using *in vivo* and *ex vivo* imaging and embryo resorption rate was calculated. RT-qPCR, Western blotting, *in situ* hybridization, and immunohistochemical studies were carried out for further *in vivo* verification. **Results** Treatment with Exo-miR-410-3p significantly reduced migration and invasion capacities of HTR-8/Svneo cells, and these inhibitory effects were effectively reversed by JSP intervention. In pregnant C57BL/6 mouse models, JSP treatment prevented Exo-miR-410-3p from reaching the maternal-fetal interface. Consistently, the embryo resorption rate was significantly lower in Exo-miR-410-3p+JSP group than in Exo-miR-410-3p alone group [(20.73±3.77)% vs (74.95±10.09)%, $P<0.01$]. JSP treatment significant reduced miR-410-3p level and upregulated TRAF6 expression in the female mouse models. **Conclusion** JSP inhibits Exo-miR-410-3p uptake by trophoblast cells, thereby promoting trophoblast EMT, migration, and invasion and reducing embryo resorption in mouse models of spontaneous abortion.

Keywords: Jianwei Shoutai Pill; spontaneous abortion; exosomal miR-410-3p; epithelial-mesenchymal transition; p38 MAPK signaling pathway

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自然流产是指妊娠28周以前发生的胚胎或胎儿丢失,是常见的生殖功能障碍,发病率约为25%^[1,2]。数据显示,全球范围内每年新发的自然流产病例高达2300万例^[3]。自然流产的病因复杂且尚未完全明确。染色体异常是最常见的病因,占自然流产病例的50%~85%^[4-6]。非遗传性、非功能性、早期的自然流产病例通常与染色

体非整倍体相关^[7]。导致自然流产的因素还包括抗磷脂综合征^[8]、遗传性血栓形成倾向^[9]和子宫结构异常^[10]。为改善自然流产女性的不良妊娠结局,临床已尝试多种干预方法,包括免疫治疗、抗凝治疗、激素补充及微量元素补充等^[11-17]。然而,目前仍缺乏公认有效的治疗方案,这给患者的身心健康及社会经济层面均造成了严峻挑战^[18, 19]。探索有效治疗措施以减少自然流产患者的妊娠终止并确保成功妊娠,对于改善生殖健康至关重要。

妊娠是一个复杂而精细的过程,人类胎盘早期发育过程中,绒毛中的细胞滋养细胞进行增殖并分化为合体滋养细胞和绒毛外滋养细胞(EVTs)。EVTs细胞的迁移和侵袭受损可导致一系列包括自然流产在内的妊娠并发症^[20-23]。上皮-间质转化(EMT)是EVTs细胞分化过程的关键步骤^[24, 25],以上皮表型丧失和间质表型获得为特征^[26]。前期研究表明,自然流产患者血清外泌体miR-410-3p可能通过靶向TNF受体相关因子6(TRAF6),抑制p38 MAPK信号通路,进而抑制HTR-8/Svneo细胞EMT、侵袭和迁移,且小鼠体内实验表明,装载miR-410-3p模拟物的牛奶外泌体(Exo-miR-410-3p)可增加小鼠胚胎吸收率^[27]。

中医学治疗自然流产具有独特优势和显著疗效^[28-33],其病因主要在于肾虚、冲任损伤、胎元不固^[33, 34]。在“肾主生殖”理论的指导下,中医妇科名家罗元恺教授

首创补肾固冲丸(后更名为滋肾育胎丸),后通过药物配伍规律研究,经临床-动物实验-专用软件模拟计算-实验验证获得减味寿胎丸(JSP)(菟丝子、桑寄生、续断),具有配伍严谨、药性平和的特点。本研究旨在探讨JSP是否能够通过抑制HTR-8/Svneo细胞摄取Exo-miR-410-3p,促进HTR-8/Svneo细胞的EMT、迁移和侵袭,进而减少自然流产。

1 材料和方法

1.1 药物制备

JSP购自广东省药材公司,并按照先前建立的流程制备(表1)^[35]。JSP溶液的配制比例为4:3:3。药材以10:1的比例用水煎煮1 h。之后,以8:1的比例用水进行第2轮煎煮1 h。将所得滤液合并,冷冻干燥24 h,得到粉末状。将冻干粉末用蒸馏水复溶以制备JSP溶液,4℃储存。

8周龄、体质量约200 g的SD大鼠(广东医学实验动物中心提供),被随机分为对照组和JSP组($n=20$)。对照组通过灌胃给予生理盐水,而JSP组则通过灌胃给予JSP(1.0 g/mL),2次/d,连续3 d。经腹主动脉采集血样,并于4℃下保存2 h,随后在4℃下以3000 r/min离心15 min。分离出的血清在56℃水浴中灭活30 min,过滤后获得对照血清或JSP含药血清,并储存于-20℃。

表1 JSP中3种组成药材的基本信息
Tab.1 Information of 3 constituent herbs in Jianwei Shoutai Pill (JSP)

Chinese name	Botanical ^a name	English name	Genus family	Part used	Batch number	Weight (g)
Tusizi	Cuscuta chinensis Lam	Cuscutae semen	Convolvulaceae	Seed	150701	20
Sangjisheng	Taxillus sutchuenensis var. duclouxii (Lecomte) H.S.Kiu	Herba taxilli	Loranthaceae	Stem	160601	15
Xudian	Dipsacus L.	Dipsaci radix	Caprifoliaceae	Stem	140705511	15

^a The plant name has been checked with<http://www.worldfloraonline.org>. Accessed on: Jun 06, 2023.

1.2 高效液相色谱-质谱联用(HPLC-MS)

通过HPLC-MS对JSP冻干粉或JSP含药血清进行定性和定量分析。将0.1 g JSP冻干粉与1000 μL 80%甲醇和研磨珠混合。将混合物研磨5 min,然后涡旋10 min。4℃条件下20 000×g离心10 min,用0.22 μm的滤器过滤上清液。将50 μL JSP含药血清与200 μL甲醇混合,并涡旋10 min。4℃条件下20 000×g离心10 min,收集上清液。HPLC-MS分析鉴定JSP冻干粉和JSP含药血清中的关键化合物,其保留时间与标准品或数据库中的相应色谱峰一致。

1.3 透射电子显微镜(TEM)

TEM用于确定外泌体的形态。首先,将外泌体重悬于2%多聚甲醛溶液中(50~100 μL)。然后,将5 μL外泌体悬液滴加到Formvar-carbon 载样铜网上。使用

镊子将铜网置于铺有石蜡膜的PBS液滴上。将铜网转移到1%戊二醛液滴(50 μL)中5 min,随后分别滴加草酸铈(50 μL)和甲基纤维素(50 μL)液滴各5 min。所有操作均在冰上进行。在空气中干燥5 min后,使用TEM获取图像。

1.4 纳米颗粒追踪分析(NTA)

使用ZetaView PMX 110和ZetaView 8.5软件,通过NTA技术测量外泌体的大小。用去离子水清洗样本池,用聚苯乙烯微球(110 nm)校准仪器,用PBS清洗样本池,再用PBS稀释外泌体,在11个位置记录和分析NTA测量值,温度保持在27.68℃。

1.5 细胞培养

人滋养层细胞系HTR-8/Svneo细胞使用含有10%胎牛血清和1%青霉素-链霉素的RPMI 1640培养基培

养,于37℃、5% CO₂条件下生长。使用胰蛋白酶对细胞进行消化,以便传代。

1.6 Western blotting

在外泌体或细胞中加入适量裂解液,冰上裂解30 min,BCA法检测蛋白浓度后变性,SDS-PAGE电泳后转膜,封闭,一抗4℃孵育过夜,二抗室温孵育1.5 h,ECL化学发光液显影法检测蛋白条带,Image Lab软件分析灰度值。所用一抗如下:CD63、CD81、TSG101 (Abcam,1:1000),TRAF6 (Affinity,1:1000),E-cadherin (1:20000)、N-cadherin (1:2000)、Vimentin (1:20000)、GAPDH (1:5000) (Proteintech),p38 MAPK、p-p38 (Cell Signaling Technology,1:1000)。二抗为山羊抗兔IgG(H+L) (Proteintech,1:5000)。

1.7 牛奶外泌体负载miR-410-3p

按照文献[35-37]方法,使用电穿孔技术将miR-410-3p模拟物递送至牛奶外泌体中。将miR-410-3p模拟物及其阴性模拟对照离心后,溶于DEPC水,然后加入牛奶外泌体中,混合后置于电穿孔杯中。使用特定参数(400 V,200 Ω,125 μF)进行电穿孔。之后,将混合物置于冰上冷却,过滤,并储存在-80℃。

1.8 RT-qPCR

按照制造商说明书,使用MagZol Reagent分离总RNA。对于miRNA表达分析,使用All-in-One™ miRNA First-Strand cDNA Synthesis试剂盒进行逆转录步骤,然后使用All-in-One™ miRNA qPCR试剂盒进行RT-qPCR。使用U6进行标准化。采用2^{-ΔΔCt}法计算相对miRNA的水平。实验引物均由上海生工生物工程有限公司合成。

1.9 细胞摄取外泌体

使用PKH26荧光染料标记Exo-Ctrl或Exo-miR-410-3p。将荧光外泌体与HTR-8/Svneo细胞共培养。孵育48 h后,使用含4',6-二脒基-2-苯基吡啶(DAPI)的抗荧光衰减封片剂对各组细胞核进行染色。然后使用IX71荧光显微镜显示并记录各组的荧光图像。

1.10 免疫荧光染色

经过适当处理的HTR-8/Svneo细胞用4%多聚甲醛固定30 min。随后,在室温下用0.3% Triton X-100处理30 min。为防止非特异性结合,在室温下用10%血清作为封闭剂孵育1 h。然后将细胞在孵育一抗4℃过夜。次日,使用荧光染料偶联的兔源二抗孵育细胞,并使用DAPI进行封片。使用荧光显微镜检查所得样本,并使用Image J测量阳性染色细胞所占区域的荧光强度。对于免疫荧光染色,使用以下兔源一抗:TRAF6 (1:500)、E-cadherin (1:500)、N-cadherin (1:500)、Vimentin (1:500)及p-p38 (1:800)。随后,使用相应的荧光二抗进行孵育:CoraLite™488-标记的山羊抗兔IgG (H+L) (1:500)用于检测E-cadherin、N-cadherin、Vimentin、

p-p38(绿色荧光),CoraLite™594-标记的山羊抗兔IgG (H+L) (1:500)用于检测TRAF6(红色荧光)。

1.11 CCK-8检测

将HTR-8/Svneo细胞接种于96孔板中,并用不同浓度的JSP含药血清或对照血清孵育,持续不同的时间间隔。按照制造商说明,使用CCK-8试剂盒测定细胞活力。酶标仪检测吸光度 $A_{450\text{nm}}$ 。细胞增殖率(%)= $[(A_{\text{实验组}}-A_{\text{空白组}})/(A_{\text{对照组}}-A_{\text{空白组}})]\times 100\%$ 。

1.12 划痕实验

细胞种板之前在6孔板各孔的底部每间隔0.5~1 cm划1条横线,共划3条横线,消化对数生长期的HTR-8/Svneo细胞并进行计数,以 $1\times 10^5/\text{mL}$ 的密度接种至6孔板中,按不同分组条件处理,细胞融合至约90%时,用200 μL枪头垂直划痕,PBS洗涤后,显微镜下观察各组细胞的迁移距离并拍照,48 h后再次观察并拍照,Image J软件分析。

1.13 Transwell实验

用RPMI 1640培养基稀释基质胶,轻柔混匀后取80 μL均匀铺于Transwell小室的上室,37℃孵育4 h使其凝固,消化对数生长期的HTR-8/Svneo细胞并进行计数,按不同分组条件处理后消化细胞,使用含有2%胎牛血清的RPMI 1640培养基制备细胞悬液,按 $1\times 10^5/\text{mL}$ 的密度接种于Transwell小室的上室,下室加入600 μL含有20%胎牛血清的RPMI 1640培养基,置于37℃、5% CO₂培养箱中培养48 h,PBS洗涤3次,4%多聚甲醛固定,甲醇透化,结晶紫染色,显微镜下观察并拍照,Image J软件分析。

1.14 瞬时转染miR-410-3p mimic和miR-410-3p inhibitor

将mimic Control、miR-410-3p mimic、inhibitor Control、miR-410-3p inhibitor粉末于4℃离心机以12 000 r/min离心2 min,用250 μL DEPC水溶解,配制成20 μmol/L的储存液,分装保存,每管冻融不超过5次。

消化对数生长期的HTR-8/Svneo细胞并进行计数,以 $1\times 10^5/\text{mL}$ 的密度接种至6孔板中,细胞融合至70%~80%时开始转染实验。对于每个转染样品,准备2个1.5 mL无酶EP管,分别设置为A管和B管,冰上进行以下操作:mimic Control和miR-410-3p mimic转染浓度为50 nmol/L,A管:500 μL Opti-MEM培养基+2.5 μL mimic Control/miR-410-3p mimic,B管:500 μL Opti-MEM培养基+7.5 μL Lipofectamine试剂,A管和B管分别室温孵育5 min后混合孵育15 min形成转染复合物。inhibitor Control和miR-410-3p inhibitor转染浓度为100 nmol/L,A管:500 μL Opti-MEM培养基+5 μL inhibitor Control/miR-410-3p inhibitor,B管:500 μL Opti-MEM培养基+10 μL Lipofectamine试剂,

A管和B管分别室温孵育5 min后混合孵育15 min形成转染复合物。取出6孔板,吸弃旧培养基,用PBS轻柔洗涤孔板中的细胞,加入1 mL转染复合物,置于37℃、5% CO₂培养箱中培养6~8 h后更换含有10%胎牛血清的RPMI 1640培养基,24~48 h后进行后续实验。

1.15 瞬时转染Si TRAF6

将Si Control、Si TRAF6-1、Si TRAF6-2、Si TRAF6-3粉末于4℃离心机以12 000 r/min离心2 min,用250 μL DEPC水溶解,配制成20 μmol/L的储存液,分装保存,每管冻融不超过5次。消化对数生长期的HTR-8/Svneo细胞并进行计数,以 1×10^5 /mL的密度接种至6孔板中,细胞融合至70%~80%时开始转染实验。对于每个转染样品,准备2个1.5 mL无酶EP管,分别设置为A管和B管,冰上进行以下操作,转染浓度为50 nmol/L, A管:500 μL Opti-MEM培养基+2.5 μL Si Control/Si TRAF6-1/Si TRAF6-2/Si TRAF6-3, B管:500 μL Opti-MEM培养基+7.5 μL Lipofectamine试剂, A管和B管分别室温孵育5 min后混合孵育15 min形成转染复合物。

取出6孔板,吸弃旧培养基,用PBS轻柔洗涤孔板中的细胞,加入1 mL转染复合物,置于37℃、5% CO₂培养箱中培养6~8 h后更换含有10%胎牛血清的RPMI-1640培养基,24~48 h后进行后续实验筛选出转染效率最佳的Si TRAF6,进行后续实验。

1.16 构建TRAF6过表达慢病毒(LV TRAF6)稳转细胞株

前期稳定株筛选实验筛选出最佳感染条件:MOI=50, Normal+HitransG A, 干预时间为72 h, Puromycin工作浓度为1 μg/mL, 干预时间为48 h。消化对数生长期的HTR-8/Svneo细胞并进行计数,以 1×10^5 /mL的密度接种至6孔板中,细胞融合度20%~30%时开始转染实验。LV Control组:加入100 μL/孔LV Control病毒、900 μL含有10%胎牛血清的RPMI 1640培养基; LV TRAF6组:加入100 μL/孔LV TRAF6病毒、40 μL HitransG A感染增强液和860 μL含有10%胎牛血清的RPMI 1640培养基; 12 h后更换含有10%胎牛血清的RPMI 1640培养基。

72 h后,加入浓度为1 μg/mL的Puromycin, 干预48 h后, LV Control组的细胞全部死亡, LV TRAF6组剩下的细胞则为阳性细胞。将Puromycin浓度减至维持浓度(筛选浓度的1/2~1/4), 继续对感染后的细胞进行筛选和传代, 同时收集细胞进行PCR或Western blotting鉴定, 并将鉴定结果正常的细胞冻存保种。

1.17 动物实验

8周龄雌性C57BL/6小鼠和雄性BALB/c小鼠(广

东省医学实验动物中心), 分为4组:模型组、模型+Exo-miR-410-3p组、模型+Exo-miR-410-3p+黄体酮(P)组和模型+Exo-miR-410-3p+JSP组($n=5$)。Exo-miR-410-3p通过尾静脉注射给药; 而黄体酮(7.56 mg/kg)和JSP(2.52 g/kg)则通过灌胃给予。雌雄小鼠的交配比例为2:1。首先确定预实验的浓度梯度。从检测到阴道栓当天(第0天)开始, 每3 d注射1次Exo-miR-410-3p或PBS(180 μg)。每天灌胃给予黄体酮或JSP。第11天, 处死小鼠以计算胚胎吸收率并测量组织内miR-410-3p和TRAF6的水平。动物实验经广州中医药大学第一附属医院实验动物伦理委员会批准[伦理批号: S-BPM-5-002-01(01)], 并严格按照机构指南进行。

1.18 荧光标记外泌体的小动物成像以及体内外追踪

最后1次尾静脉注射后, 小动物活体成像检测Exo-Ctrl和Exo-miR-410-3p在小鼠血液循环和器官的生物分布, 获得不同器官的体内荧光图像和离体荧光图像, 使用小动物活体成像仪和Living Image® 4.5.2软件对荧光强度进行量化, 以评估DiR标记的外泌体的分布情况。

1.19 免疫组织化学(IHC)

石蜡切片脱蜡后, 枸橼酸抗原修复, PBS洗涤, 3%过氧化氢溶液室温孵育20 min, PBS洗涤, 室温血清封闭30 min, 4℃孵育一抗过夜, PBS洗涤, 室温孵育二抗1 h, PBS洗涤, DAB显色, 苏木素复染细胞核, 脱水透明后封片。使用Pannoramic DESK扫描仪扫描IHC切片, 并通过CaseViewer进行分析。免疫组化染色中, 使用的兔源一抗为TRAF6(1:100), 使用二抗为HRP标记的山羊抗兔IgG(H+L)二抗(1:1000)。

1.20 原位杂交

组织切片经二甲苯脱蜡、梯度乙醇水化后, 用DEPC水洗涤。于抗原修复液中煮沸10 min, 自然冷却; 随后用蛋白酶K 37℃消化15 min。PBS洗涤后, 切片置于3% H₂O₂-甲醇溶液中室温封闭20 min。加入预杂交液37℃孵育30 min, 然后更换为含500 nmol/L miR-410-3p探针的杂交液, 40℃孵育过夜。经柠檬酸钠盐溶液洗涤后, 室温封闭30 min, 加入小鼠抗地高辛标记过氧化物酶(1:1000), 4℃孵育过夜。DAB显色、苏木精复染后, 梯度乙醇脱水、二甲苯透明, 封片。使用Pannoramic DESK扫描仪采集图像, CaseViewer软件进行分析。

1.21 统计学分析

采用SPSS 26.0软件进行统计分析。所有实验均独立重复3次, 计量资料以均数±标准差表示。对数据进行正态性检验(Shapiro-Wilk检验)和方差齐性检验(Levene检验)。多组间比较采用单因素方差分析; 若方

差齐,两两比较采用LSD-*t*检验;若方差不齐,则采用Games-Howell检验。两组间比较采用独立样本*t*检验(方差齐)或Welch's *t*检验(方差不齐)。以*P*<0.05为差异有统计学意义。

2 结果

2.1 JSP冻干粉末和JSP含药血清的化学特征和物质基础
采用HPLC-MS鉴定JSP冻干粉末的主要化学成分(图1A,B)。根据精确质量、色谱行为、质谱/质谱数

据和裂解规律,通过与既往研究和mzCloud数据库进行比较,鉴定或初步表征了包括间苯三酚、玉叶金花苷酸、夹竹桃麻素等在内的20种质量控制成分(表2、3)。使用HPLC-MS鉴定JSP含药血清的主要化学成分(图1C,D)。根据精确质量、色谱行为、质谱/质谱数据和裂解规律,通过与先前的研究和mzCloud数据库进行比较,鉴定或初步表征了19种质量控制成分,包括亚油酸、 γ -亚麻酸乙酯、1-硬脂酰甘油等(表4、5)。

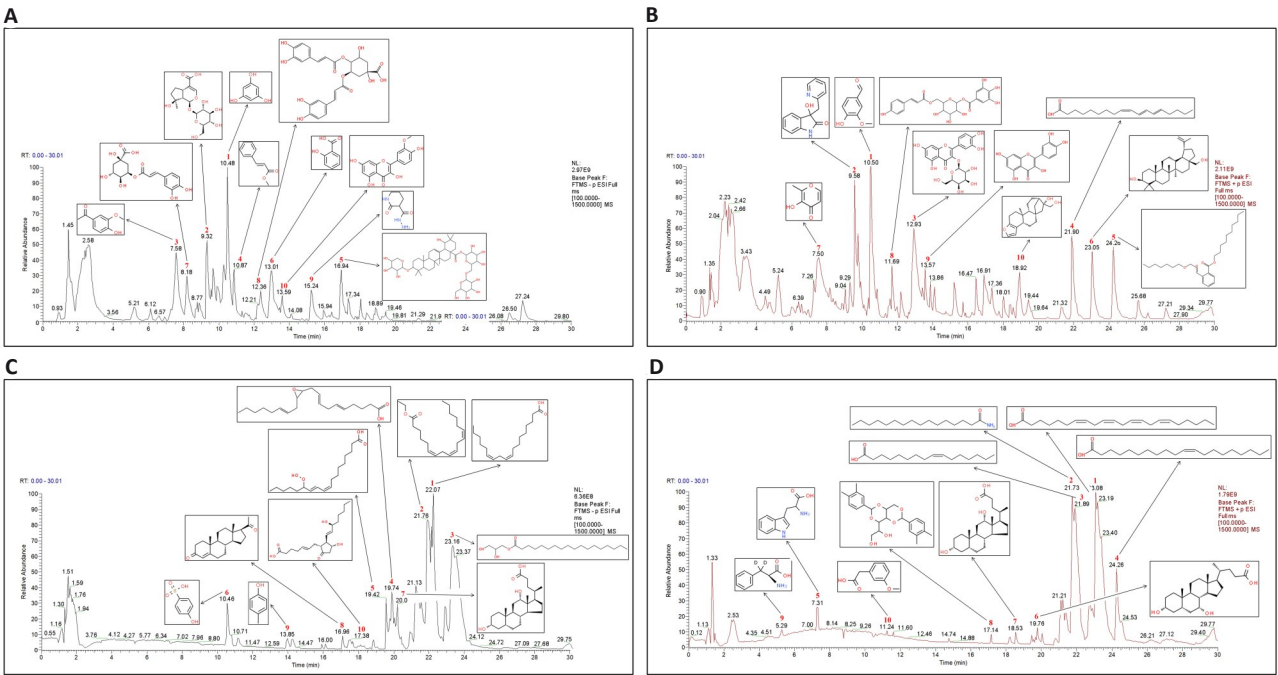


图1 JSP冻干粉和JSP含药血清的化学成分和物质基础
Fig. 1 Chemoprofile and material basis of JSP lyophilized powder and JSP-mediated rat serum. **A:** Peak number and chemical characterization of JSP lyophilized powder in negative ion mode. **B:** Peak number and chemical characterization of JSP lyophilized powder in positive ion mode. **C:** Peak number and chemical characterization of JSP-containing serum in negative ion mode. **D:** Peak number and chemical characterization of JSP-containing serum in positive ion mode.

表2 负离子模式下HPLC-MS分析得到的JSP冻干粉末中的10大主要成分
Tab.2 Top 10 ingredients of JSP lyophilized powder by HPLC-MS analysis under negative ion mode

No.	Identification	Formula	RT (min)	[M-H] ⁻ (m/z)			MS2
				Measured	Predicted	Δ (ppm)	
1	Phloroglucinol	C6H6O3	10.49	127.03892	126.03166	-0.304	109.02865, 99.04446
2	Mussaenosidic acid	C16H24O10	9.32	375.12921	376.13664	1.063	213.07642, 195.06569
3	Apocynin	C9H10O3	7.66	167.07027	166.06299	-0.025	167.07004, 152.04672
4	Methyl cinnamate	C10H10O2	10.91	180.10168	179.09449	-3.573	164.04254, 163.11104
5	6-O-Hexopyranosyl-1-O-[19-hydroxy-28-oxo-3-(pentopyranosyloxy)olean-12-	C47H76O18	16.94	973.5009	974.50825	0.493	928.50079, 927.49738
6	Salicylic acid	C7H6O3	12.96	137.02315	138.03042	-9.200	138.02663, 138.01686
7	Neochlorogenic acid	C16H18O9	8.18	353.08746	354.09470	-1.082	191.05544, 179.03421
8	4,5-Dicaffeoylquinic acid	C25H24O12	12.38	515.11902	516.12623	-1.051	354.09219, 353.08780
9	2-oxopiperidine-3-carbohydrazide	C6H11N3O2	15.25	156.08057	157.08784	-0.557	155.07317, 128.03448
10	Isorhamnetin	C16H12O7	13.65	317.06503	316.05776	-1.729	317.06497, 302.04166

表3 正离子模式下HPLC-MS分析所得JSP冻干粉的10大主要成分
Tab.3 Top 10 ingredients in JSP lyophilized powder by HPLC-MS analysis under positive ion mode

No.	Identification	Formula	RT (min)	[M-H] ⁺ (m/z)			MS2
				Measured	Predicted	Δ (ppm)	
1	Vanillin	C8H8O3	10.57	153.05446	152.04718	-1.066	136.07547, 135.11678
2	3-hydroxy-3-(2-pyridylmethyl)indolin-2-one	C14H12N2O2	9.59	241.09671	240.08944	-1.834	224.08940, 223.08618
3	Hyperoside	C21H20O12	12.85	465.10165	464.09481	-1.427	304.05289, 303.04953
4	α-Eleostearic acid	C18H30O2	21.85	279.23154	278.22430	-1.021	279.23132, 279.09326
5	Octyl decyl phthalate	C26H42O4	24.28	441.29675	440.28948	-1.272	315.15570, 203.17888
6	Betulin	C30H50O2	23.05	411.36124	410.35400	-1.438	397.26166, 359.24600
7	Maltol	C6H6O3	7.52	127.0390	126.03172	0.244	127.03897, 109.02870
8	6-O-[(2E)-3-(4-Hydroxyphenyl)-2-propenoyl]-1-O-(3,4,5-trihydroxybenzoyl)hexopyranose	C22H22O12	11.73	477.10422	478.11149	0.961	477.10388, 433.11270
9	Quercetin	C15H10O7	13.57	303.04919	302.04192	-1.854	285.03885, 275.05548
10	Cafestol	C20H28O3	18.92	317.20819	316.20091	-1.739	299.19794, 271.20505

表4 负离子模式下HPLC-M分析得到的含JSP血清10大主要成分
Tab.4 Top 10 ingredients of JSP-medicated rat serum by HPLC-MS analysis under negative ion mode

No.	Identification	Formula	RT(min)	[M-H] ⁻ (m/z)			MS2
				Measured	Predicted	Δ(ppm)	
1	Linoleic acid	C18H32O2	22.15	325.23828	280.24003	-0.715	279.23294, 59.01225
2	γ-Linolenic acid ethyl ester	C20H34O2	21.68	313.27289	312.26561	-1.761	257.24731, 239.23659
3	1-Stearoylglycerol	C21H42O4	23.04	381.29657	358.30739	-2.560	341.30453, 285.27875
4	(±)11(12)-EET	C20H32O3	19.75	319.22751	320.23479	1.717	301.21756, 257.22751
5	(±)13-HpODE	C18H32O4	19.39	295.22638	294.21925	-0.816	295.22440, 295.18799
6	4-Phenolsulfonic acid	C6H6O4S	10.45	172.99033	173.99760	3.218	93.03314, 79.95588
7	Deoxycholic Acid	C24H40O4	20.02	391.28513	392.29241	-0.648	365.30621, 347.29520
8	Progesterone	C21H30O2	16.98	315.23166	314.22438	-0.665	315.23148, 297.22113
9	4-Methylphenol	C7H8O	13.86	107.04849	108.05577	5.090	67.50848
10	5-trans-Prostaglandin E2	C20 H32O5	17.37	335.22116	334.21403	-2.094	335.22006, 317.21027

表5 正离子模式下HPLC-MS分析所得含JSP血清的10大主要成分
Tab.5 TOP 10 ingredients of JSP-medicated rat serum by HPLC-MS analysis under positive ion mode

No.	Identification	Formula	RT(min)	[M-H] ⁺ (m/z)			MS2
				Measured	Predicted	Δ(ppm)	
1	Adrenic acid	C22H36O2	23.00	331.26413	332.27136	-0.503	71.01227, 59.00959
2	Hexadecanamide	C16H33NO	21.56	256.26300	255.25574	-1.879	256.26297, 102.09174
3	Palmitoleic acid	C16H30O2	21.91	253.21693	254.22421	-1.453	59.01217, 53.94514
4	11(Z)-Eicosenoic acid	C20H38O2	24.23	309.28006	310.28733	0.489	309.17767, 104.95240
5	DL-Tryptophan	C11H12N2O2	7.28	188.07050	204.08978	-0.469	188.07042, 170.05983
6	Chenodeoxycholic Acid	C24H40O4	19.86	437.29034	438.29762	1.255	409.29636, 391.28537
7	Deoxycholic Acid	C24H40O4	18.52	391.28519	392.29249	-0.648	347.29453, 343.26413
8	Bis(4-ethylbenzylidene)sorbitol	C24H30O6	17.13	415.21078	414.20352	-1.736	135.08035, 119.08560
9	L-Phenylalanine	C9H11NO2	5.27	149.05977	148.05242	-5.921	131.04912, 120.08086
10	3-Methoxyphenylacetic acid	C9H10O3	11.28	165.05475	166.06213	-5.203	165.05470, 165.01831

2.2 Exo-Ctrl和Exo-miR-410-3p的表征与鉴定

对商品化牛奶外泌体进行鉴定,TEM呈现出典型的杯状形态(图2A),直径分布在30~150 nm(图2B)。Western blotting显示外泌体中富含特异性标记物CD81、CD63和TSG101(图2C)。电穿孔后得到Exo-Ctrl和Exo-miR-410-3p。RT-qPCR显示,Exo-miR-

410-3p中miR-410-3p的表达增加($P<0.01$,图2D)。NTA显示,Exo-Ctrl或Exo-miR-410-3p的直径分布在30~150 nm(图2E)。进一步将HTR-8/Svneo细胞与PKH26标记的Exo-Ctrl或Exo-miR-410-3p共孵育48 h,发现HTR-8/Svneo细胞可有效摄取PKH26标记的Exo-Ctrl和Exo-miR-410-3p(图2F)。

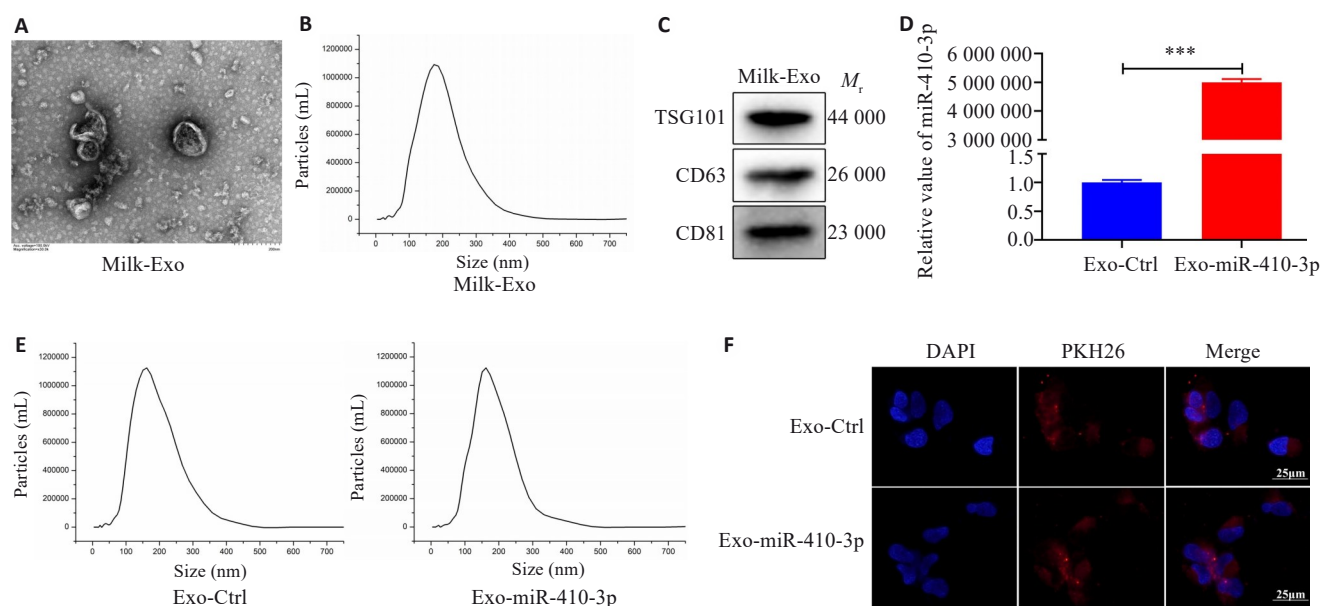


图2 Exo-Ctrl和Exo-miR-410-3p的表征和鉴定

Fig.2 Characterization and identification of Exo-Ctrl and Exo-miR-410-3p. A: TEM image of milk exosomes (Scale bar=200 nm). B: NTA of the size distribution of milk exosomes. C: Protein levels of CD63, CD81, and TSG101 in milk exosomes measured by Western blotting. D: Expression level of miR-410-3p in Exo-Ctrl or Exo-miR-410-3p measured by RT-qPCR. E: NTA of the size distribution of Exo-Ctrl or Exo-miR-410-3p. F: Fluorescence image of HTR-8/Svneo cells incubated with PKH26-labeled Exo-Ctrl or Exo-miR-410-3p (red) (Scale bar=25 μ m). ($n=3$). *** $P<0.001$.

2.3 Exo-miR-410-3p抑制HTR-8/Svneo细胞EMT、迁移和侵袭

RT-qPCR显示,相比于Exo-Ctrl组,经Exo-miR-410-3p处理的HTR-8/Svneo细胞中miR-410-3p的表达升高(1.25 ± 0.29 vs 3.06 ± 0.34 , $P<0.01$,图3A)。Western blotting和免疫荧光染色显示,Exo-miR-410-3p上调E-cadherin蛋白表达,并下调TRAF6、N-cadherin和Vimentin蛋白表达($P<0.01$,图3B、C)。划痕实验和Transwell实验表明,相比于Exo-Ctrl组,Exo-miR-410-3p抑制了HTR-8/Svneo细胞的迁移能力[(43.68 ± 4.55)% vs (21.71 ± 6.88)%, $P<0.01$,图3D]和侵袭能力(0.96 ± 0.07 vs 0.54 ± 0.07 , $P<0.01$,图3E)。

2.4 JSP含药血清逆转Exo-miR-410-3p对HTR-8/Svneo细胞EMT、迁移和侵袭的抑制作用

CCK-8试验显示,与未处理组相比,Exo-miR-410-3p抑制了HTR-8/Svneo细胞增殖,而Ctrl血清组(5%、10%、15%)对HTR-8/Svneo细胞增殖无明显促进作用。然而,与Ctrl血清组相比,JSP含药血清(5%、10%、15%)逆转了Exo-miR-410-3p对HTR-8/Svneo细胞增殖的抑

制作用($P<0.05$,图4A),其中15%浓度时效果最为显著[(0.57 ± 0.03)% vs (0.81 ± 0.04)%, $P<0.01$]。因此,后续的实验使用15%的对照血清或JSP含药血清进行。荧光显微镜观察显示,相比于对照血清,JSP含药血清组HTR-8/Svneo细胞中PKH26的荧光强度降低($P<0.01$,图4B)。Western blotting和免疫荧光染色显示,与Ctrl血清组相比,JSP含药血清逆转了Exo-miR-410-3p对HTR-8/Svneo细胞TRAF6、E-cadherin、N-cadherin和Vimentin蛋白表达的影响(图4C、D)。此外,与Ctrl血清组相比,JSP含药血清逆转了Exo-miR-410-3p对HTR-8/Svneo细胞的迁移[(24.24 ± 2.68)% vs (42.71 ± 3.55)%, $P<0.01$,图4E]和侵袭能力的抑制作用(0.61 ± 0.06 vs 0.96 ± 0.02 , $P<0.01$,图4F)。

2.5 JSP含药血清对HTR-8/Svneo细胞p38 MAPK信号通路的影响

Western blotting和免疫荧光染色显示,与Exo-Ctrl组相比,Exo-miR-410-3p下调了p38 MAPK信号通路中的p-p38蛋白的表达($P<0.01$,图5A、C)。然而,JSP含药血清逆转了Exo-miR-410-3p引起的下调($P<0.01$,图5B、D)。

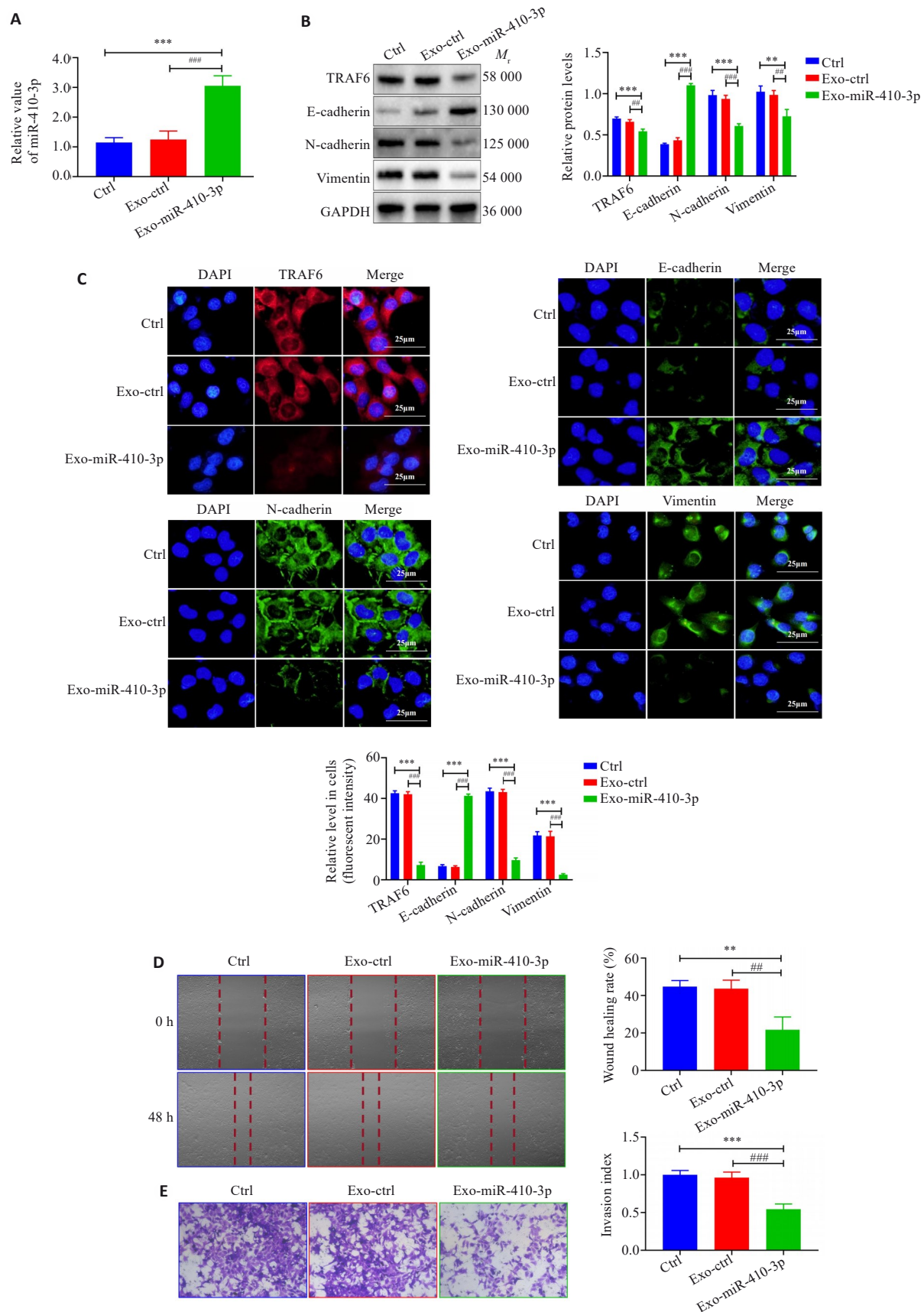


图3 Exo-miR-410-3p抑制HTR-8/Svneo细胞EMT、迁移和侵袭

Fig.3 Exo-miR-410-3p suppresses EMT, migration, and invasion of HTR-8/Svneo cells. **A**: Expression level of miR-410-3p in HTR-8/Svneo cells treated with Exo-Ctrl or Exo-miR-410-3p measured by RT-qPCR. **B**, **C**: Protein levels of TRAF6, E-cadherin, N-cadherin, and vimentin in HTR-8/Svneo cells treated with Exo-Ctrl or Exo-miR-410-3p measured by Western blotting and immunofluorescence staining. **D**, **E**: Migration and invasion capacities of HTR-8/Svneo cells treated with Exo-Ctrl or Exo-miR-410-3p determined by wound healing and Transwell assays, respectively. Representative images of migrated or invaded cells are shown (Original magnification: $\times 200$). ($n=3$). ** $P<0.01$, *** $P<0.001$; ## $P<0.01$, ### $P<0.001$.

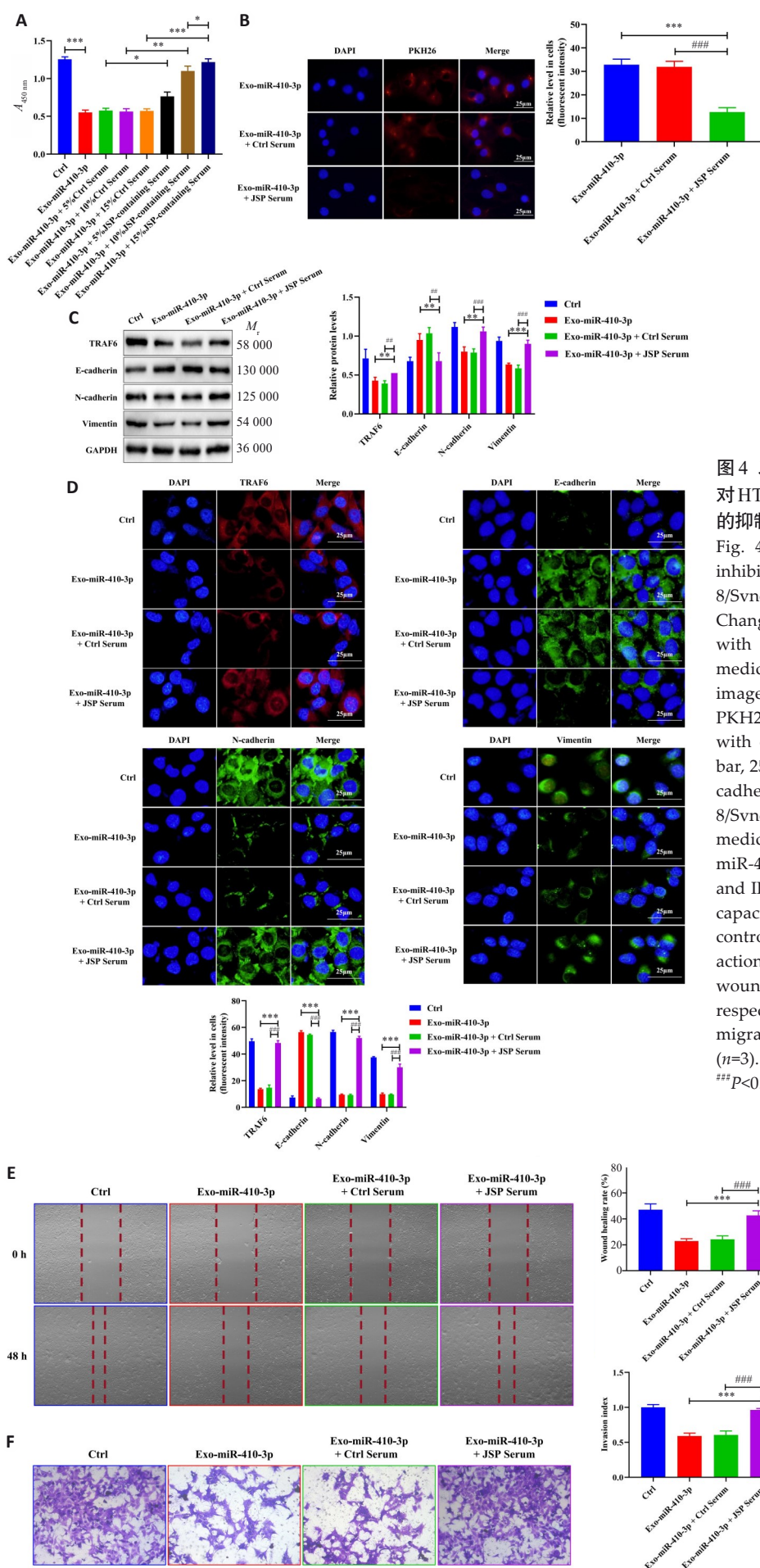


图4 JSP含药血清逆转了Exo-miR-410-3p对HTR-8/Svneo细胞的EMT、迁移和侵袭的抑制作用

Fig. 4 JSP-mediated rat serum reverses inhibitory effects of Exo-miR-410-3p on HTR-8/Svneo cell EMT, migration, and invasion. **A**: Change of HTR-8/Svneo cell viability treated with Exo-miR-410-3p, control or JSP-mediated serum for 48 h. **B**: Fluorescence image of HTR-8/Svneo cells incubated with PKH26-labeled Exo-miR-410-3p (red) treated with control or JSP-mediated serum (scale bar, 25 μ m). **C**, **D**: Protein levels of TRAF6, E-cadherin, N-cadherin, and Vimentin in HTR-8/Svneo cells treated with control or JSP-mediated serum under the action of Exo-miR-410-3p measured by Western blotting and IF staining. **E**, **F**: Migration and invasion capacities of HTR-8/Svneo cells treated with control or JSP-containing serum under the action of Exo-miR-410-3p determined by wound healing and Transwell assays, respectively. Representative images of migrated or invaded cells are shown ($\times 200$). ($n=3$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$; ## $P<0.01$, ### $P<0.001$.

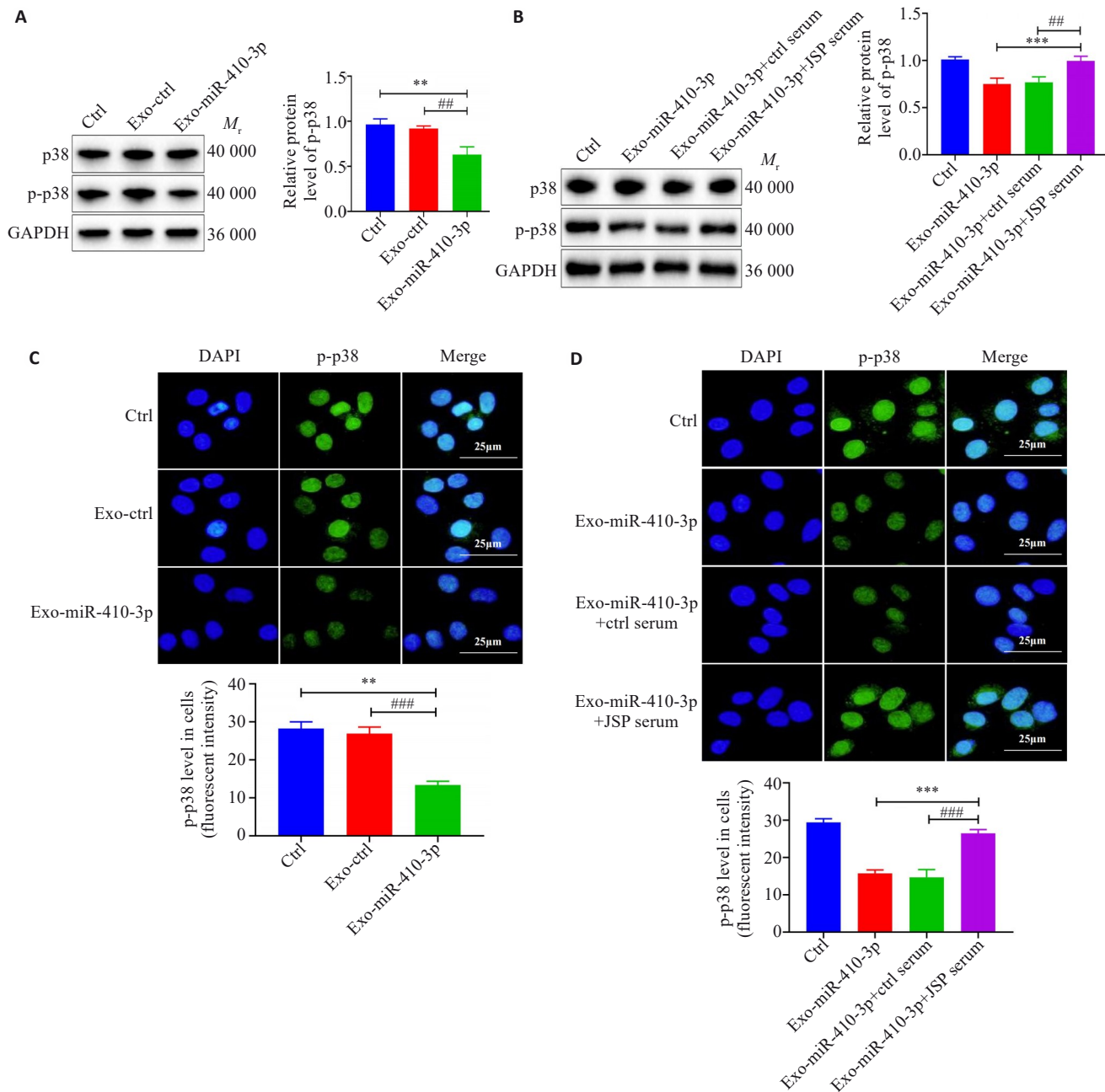


图5 JSP含药血清对HTR-8/Svneo细胞p38 MAPK信号通路的影响

Fig.5 Effect of JSP-mediated serum on the p38 MAPK signaling pathway in HTR-8/Svneo cells. **A, C**: Protein levels of p38 and p-p38 in HTR-8/Svneo cells treated with Exo-Ctrl or Exo-miR-410-3p measured by Western blotting and IF staining. **B, D**: Protein levels of p38 and p-p38 in HTR-8/Svneo cells treated with control or JSP-mediated serum under the action of Exo-miR-410-3p measured by Western blotting and IF staining. ($n=3$). ** $P<0.01$, *** $P<0.001$, ** $P<0.01$, *** $P<0.001$ vs indicated group.

2.6 功能干预实验证实JSP通过miR-410-3p/TRAF6/p38 MAPK通路发挥调控作用

Western blotting结果显示,miR-410-3p mimic上调E-cadherin表达,下调N-cadherin、Vimentin表达($P<0.05$,图7A),同时下调TRAF6和p-p38水平($P<0.05$,图6A、B),而miR-410-3p inhibitor则呈现出相反趋势。其次,敲低TRAF6(si TRAF6)后,E-cadherin升高,N-cadherin和Vimentin降低(图7B),同时p-p38降低(图6C),而过表达TRAF6(LV TRAF6)则逆转这些变化。拯救实验显示,共转染LV TRAF6可显著拮抗

miR-410-3p mimic所诱导的上述蛋白表达改变(图6D,图7C)。进一步使用p38 MAPK信号通路抑制剂SB203580干预,结果显示SB203580可阻断miR-410-3p inhibitor或LV TRAF6对相关蛋白的调控作用(图6E、F)。

2.7 JSP减少了Exo-miR-410-3p诱导的胚胎吸收

动物实验结果显示,与低剂量和中剂量相比,高剂量的JSP降低雌性小鼠血清、胎盘和子宫中miR-410-3p水平($P<0.05$,图8A~C),并增加了胎盘中TRAF6的表达(图8D),故后续实验使用高剂量JSP。小动物离体成像

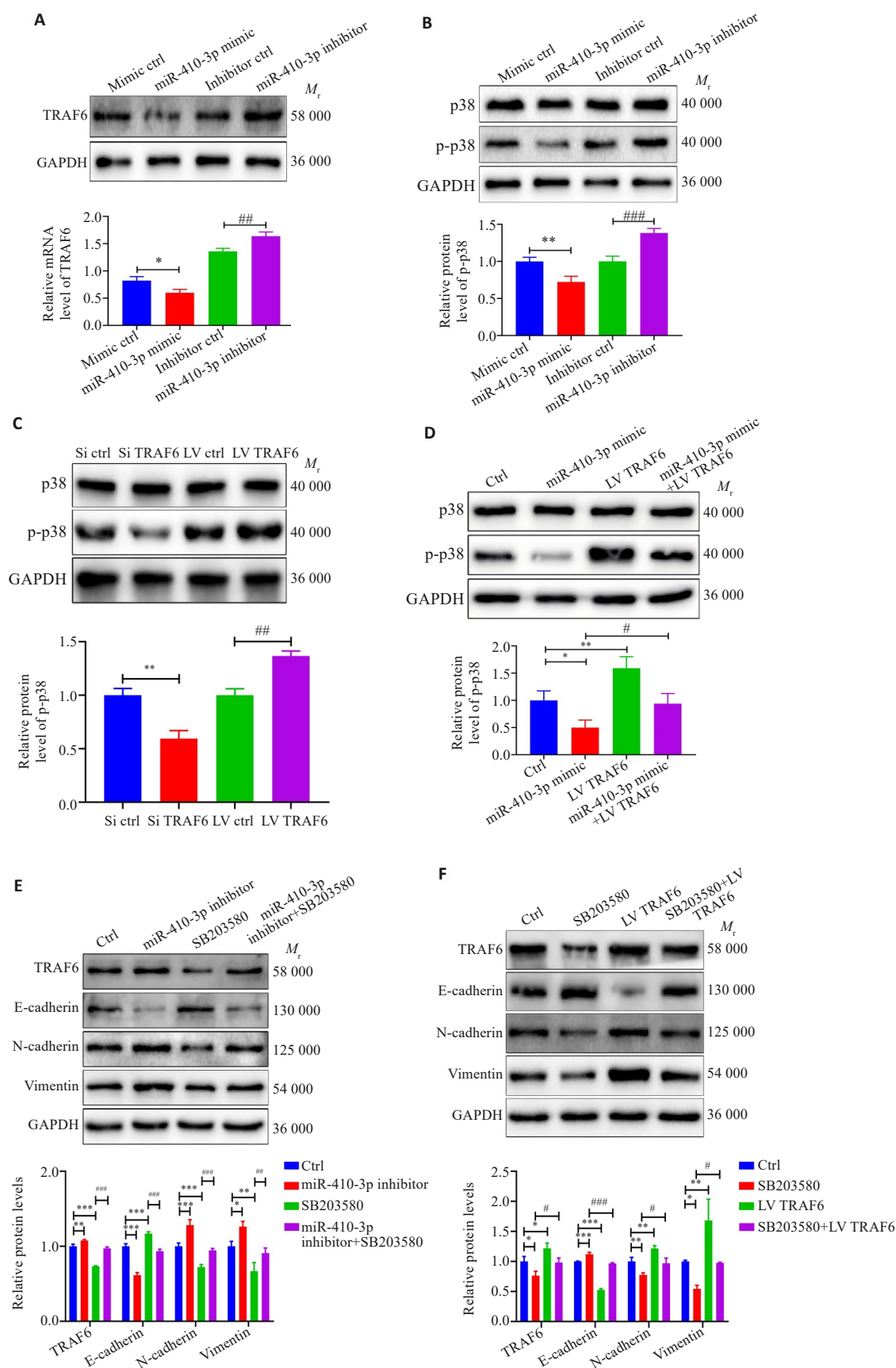


图6 miR-410-3p/TRAF6/p38 MAPK通路在JSP调控中的核心作用

Fig. 6 The core role of the miR-410-3p/TRAF6/p38 MAPK pathway in JSP-mediated regulation. **A, B:** After transfection with miR-410-3p mimic or inhibitor in HTR-8/Svneo cells, the expression of TRAF6 and p-p38 proteins was detected by Western blotting. **C:** Effect of knocking down TRAF6 (si TRAF6) or overexpressing TRAF6 (LV TRAF6) on p-p38 protein expression. **D:** Rescue experiment of co transfection of miR-410-3p mimic and LV TRAF6. **E, F:** The p38 MAPK signaling pathway inhibitor SB203580 blocks the effects of miR-410-3p inhibitor or LV TRAF6. ($n=3$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$, # $P<0.05$, ## $P<0.01$, ### $P<0.001$.

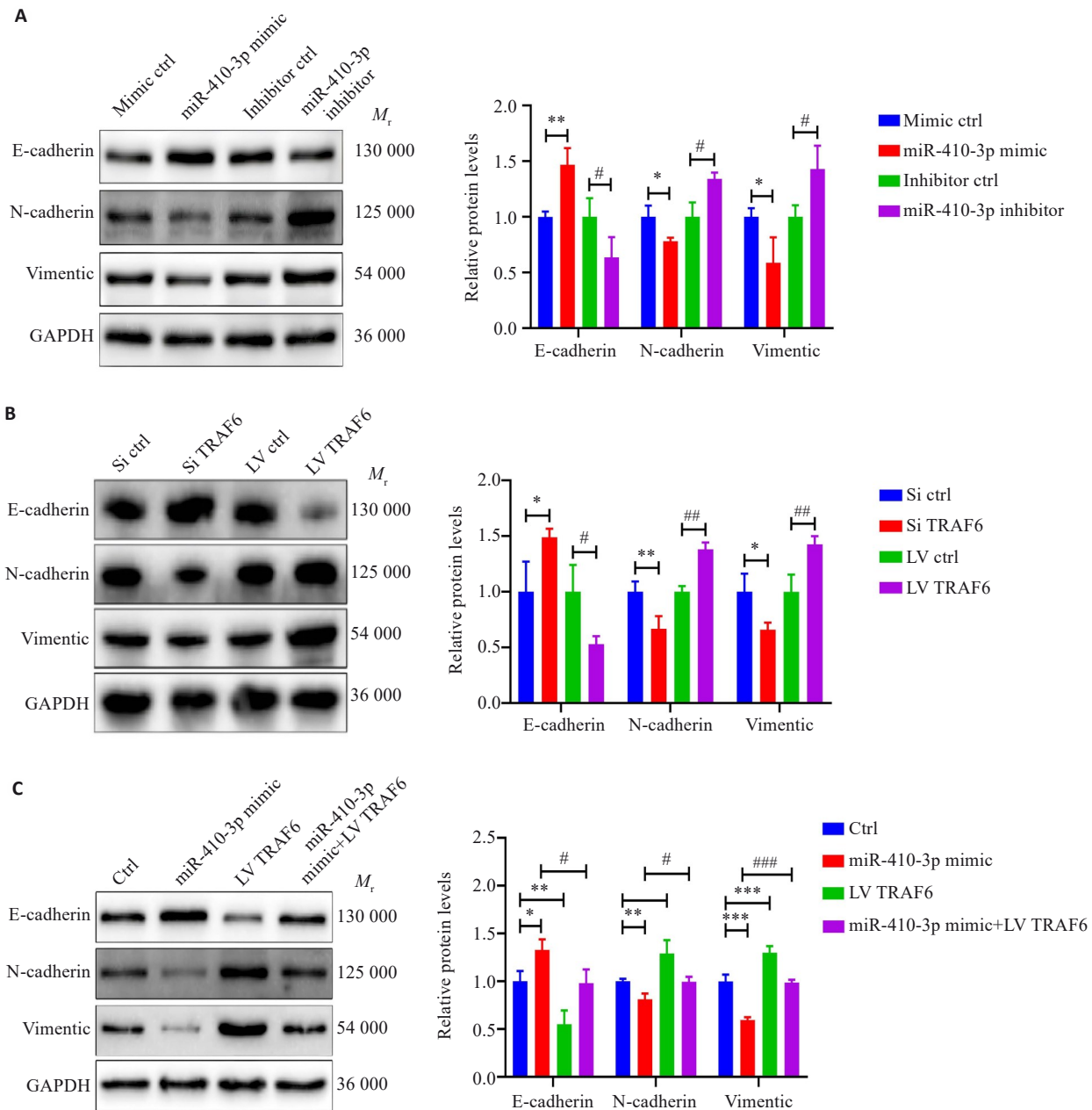


图7 不同条件下E-cadherin、N-cadherin、Vimentin蛋白的表达情况

Fig.7 Expression of E-cadherin, N-cadherin, and vimentin proteins under different conditions. **A:** After transfection of miR-410-3p mimic or inhibitor in HTR-8/Svneo cells, the protein expression of E-cadherin, N-cadherin, and vimentin was detected by Western blotting. **B:** Effect of knocking down TRAF6 (si TRAF6) or overexpressing TRAF6 (LV TRAF6) on the expression of E-cadherin, N-cadherin, and Vimentin proteins. **C:** Effect of co transfection of miR-410-3p mimic and LV TRAF6 on the expression of E-cadherin, N-cadherin, and vimentin proteins. ($n=3$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$, # $P<0.05$, ## $P<0.01$, ### $P<0.001$.

结果显示,Exo-miR-410-3p除了在雌性小鼠肺、肝和脾中积累外,在胚胎中也有显著积累。与Model+Exo-miR-410-3p组相比,Model+Exo-miR-410-3p+JSP组小鼠胚胎中Exo-miR-410-3p的积累减少($P<0.05$,图9A)。与Model+Exo-miR-410-3p组相比,Model+Exo-miR-410-3p+JSP组小鼠血清、胎盘和子宫中miR-410-3p水平降低($P<0.05$,图9B~D),并且Model+Exo-miR-410-3p+JSP组小鼠胎盘中TRAF6表达增加(图9F)。此外,Model+Exo-miR-410-3p组胚胎吸收率也高于

Model+Exo-miR-410-3p+JSP组($P<0.01$,图9E)。原位杂交和IHC检测显示,Model+Exo-miR-410-3p组miR-410-3p的水平升高,通过JSP治疗后,miR-410-3p的水平降低而TRAF6表达升高(图9G、H)。综合上述体外及体内实验结果,总结JSP治疗SA的潜在分子机制模式(图10)。

3 讨论

本研究外实验发现,JSP可能通过抑制HTR-8/

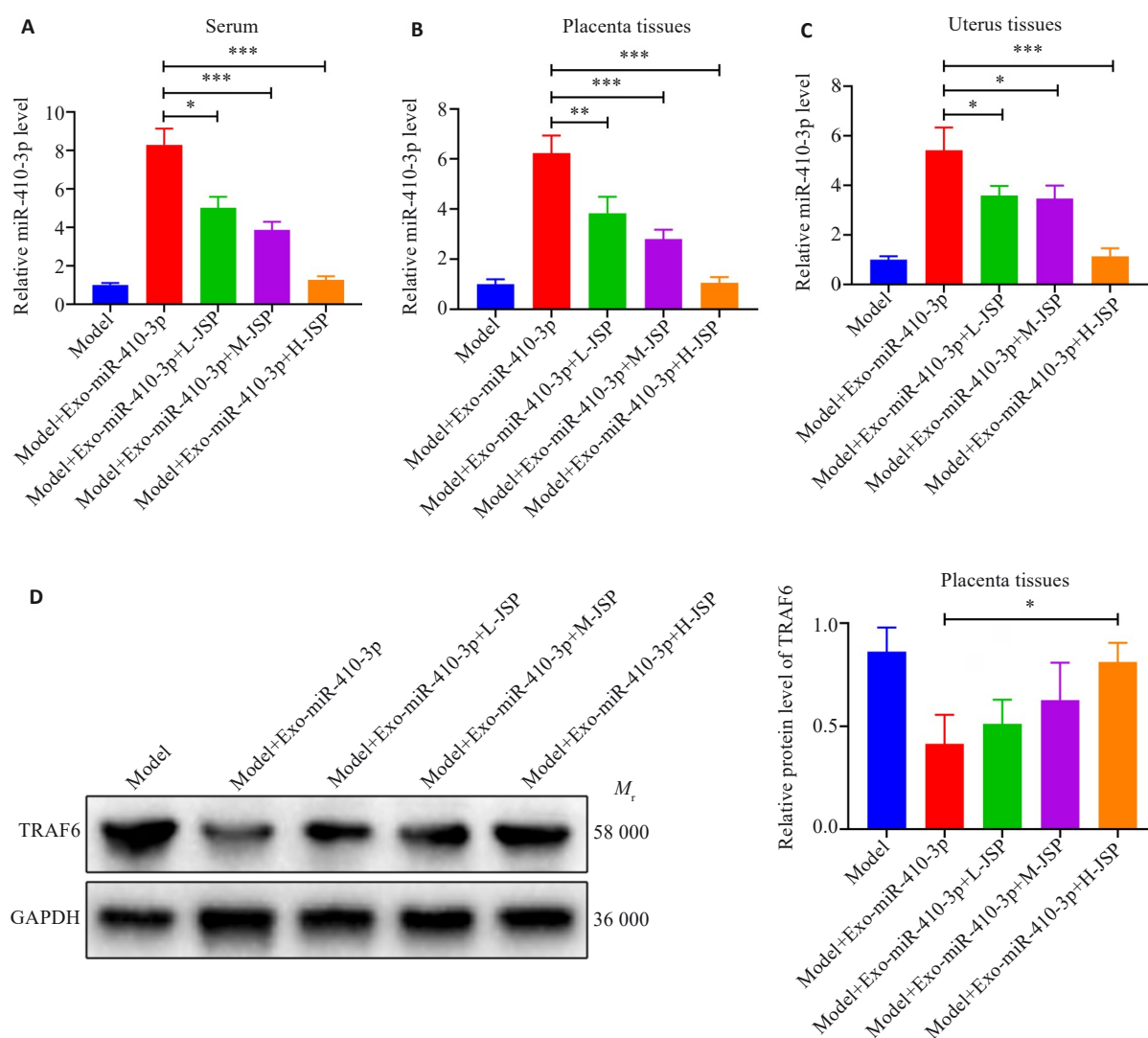


图8 高剂量的JSP降低雌性小鼠血清、胎盘和子宫中miR-410-3p的水平,并增加了胎盘中TRAF6的表达

Fig. 8 High doses of JSP reduces miR-410-3p in the serum, placenta, and uterus of female mice, but increases TRAF6 expression in the placenta. A-C: Level of miR-410-3p in the serum, placenta, and uterus of female mice treated with low, medium, and high doses of JSP detected by RT-qPCR ($n=5$). D: Expression level of TRAF6 protein in the placenta of female mice treated with low, medium, and high doses of JSP measured by Western blotting ($n=3$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Svneo细胞对Exo-miR-410-3p的摄取,从而促进HTR-8/Svneo细胞EMT、迁移和侵袭。体内实验发现,JSP可能通过抑制Exo-miR-410-3p穿过母胎界面到达胚胎减少小鼠胚胎吸收。因此,JSP在SA患者中具有重要的治疗作用。

EMT是上皮细胞向间充质细胞的转变关键过程^[38-40],能够调节滋养层外胚层细胞的迁移和侵袭^[41, 42]。在EMT诱导信号的作用下,上皮细胞减弱或消除细胞间粘附,并下调上皮细胞标志物的表达,进一步促进上皮细胞的迁移和侵袭^[43]。滋养层细胞迁移和侵袭能力不足与自然流产发生有关。既往研究表明,多种miRNA参与调控滋养层细胞EMT及迁移侵袭过程。例如,miR-27a-3p通过靶向USP25抑制滋养层细胞EMT进程^[23];巨噬细胞M1来源的外泌体携带miR-146a-5p和miR-146b-5p,通过抑制TRAF6表达削弱滋

养层细胞功能^[44];miR-373-3p则通过靶向CD44和RDX降低MMP-9表达,抑制细胞迁移侵袭^[45]。与上述miRNA类似,本研究中miR-410-3p也通过靶向TRAF6发挥抑制作用。然而,与既往研究不同的是,本研究提示JSP含药血清并非直接调控miR-410-3p的表达,而可能是通过抑制滋养层细胞对外泌体miR-410-3p的摄取来发挥保护作用。这一发现揭示了中药复方调控母胎界面信号交流的新机制,即通过干预外泌体摄取而非改变miRNA自身水平来影响细胞功能。因此,通过激活EMT来增强滋养层细胞的侵袭和迁移,为妊娠并发症的治疗提供了新的思路。

基于合成的miRNA递送系统存在诸多缺点,包括稳定性低、载药效率低、可扩展性差、合成难度大、靶向性不特异以及长期毒性^[46-51]。与合成纳米颗粒相比,天然的牛奶外泌体是一种生物相容性强且递送高效的药

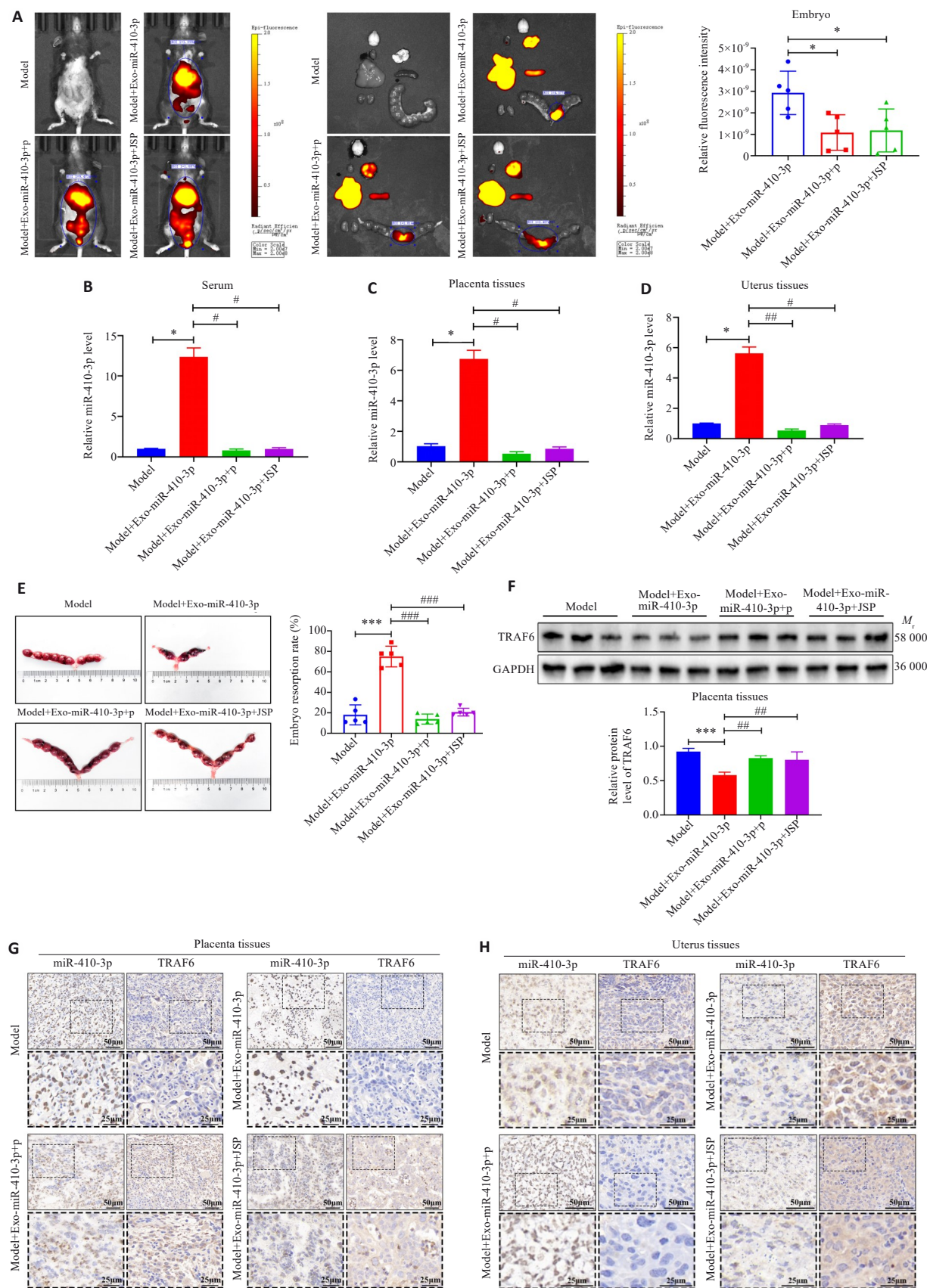


图9 JSP减少Exo-miR-410-3p诱导的胚胎吸收

Fig.9 JSP can reduce embryo resorption rate induced by Exo-miR-410-3p. **A:** *In vivo* fluorescence images of DiR labeled Exo-miR-410-3p in female mice, representative *ex vivo* fluorescence images in different organs, and the quantification of *ex vivo* fluorescence images in embryos (*n*=5). **B-D:** Expression level of miR-410-3p in the serum, placenta, and uterus of female mice detected by RT-qPCR (*n*=5). **E:** Embryo resorption rate of female mice (*n*=5). **F:** Expression level of TRAF6 protein in the placenta of female mice measured by Western blotting (*n*=3). **G, H:** Distributions of miR-410-3p and TRAF6 in the placenta and uterus of female mice detected by ISH and IHC, respectively (Scale bars=50 μm, 25 μm). **P*<0.05, ****P*<0.001, #*P*<0.05, ##*P*<0.01, ###*P*<0.001.

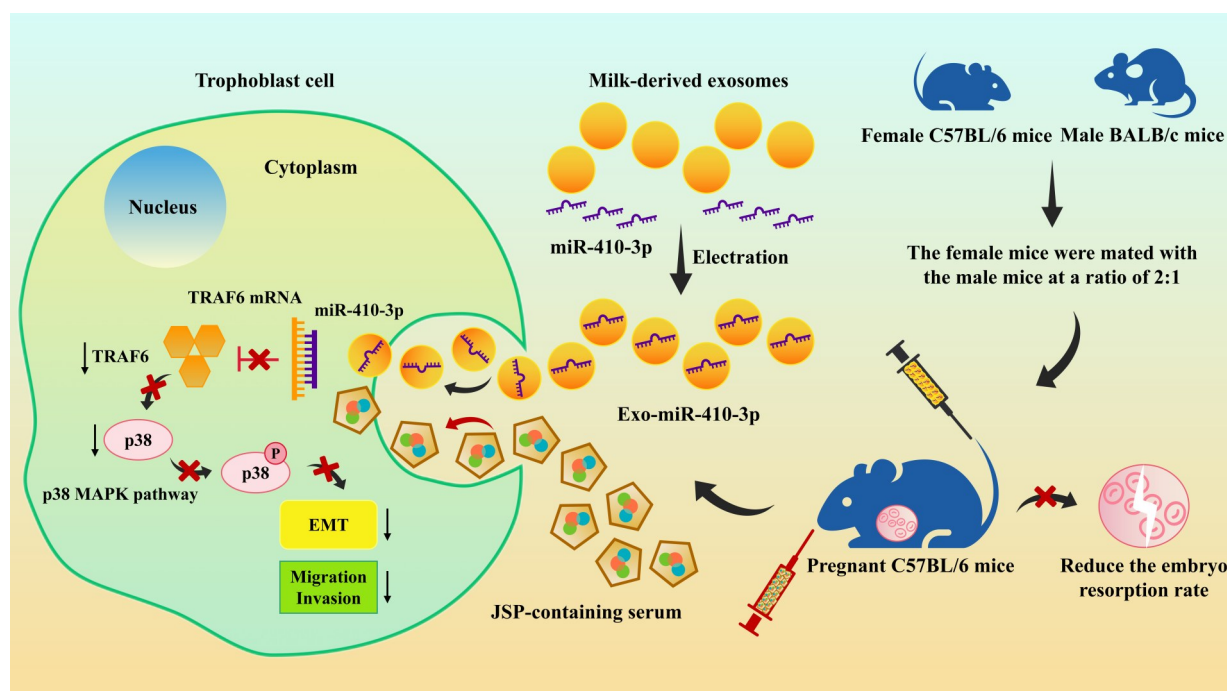


图10 JSP通过抑制Exo-miR-410-3p摄取调控滋养细胞EMT、迁移及侵袭减少SA的机制模式图

Fig.10 Schematic diagram illustrating the mechanism by which JSP reduces SA by inhibiting Exo-miR-410-3p uptake to regulate trophoblast EMT, migration, and invasion.

物递送系统^[52, 53]。牛奶外泌体有易摄取、优越的生物分布和高效递送等优势,它们能有效地将内源性RNA递送到受体细胞中,使其成为siRNA递送的潜在系统^[54]。许多研究采用牛奶外泌体作为递送载体,例如负载miR-31-5p模拟物的牛奶外泌体表现出易被细胞摄取、抗降解性、体外改善内皮细胞功能,以及体内刺激血管生成和伤口愈合的作用^[55]。而在本研究中,使用牛奶外泌体作为miR-410-3p模拟物的载体,装载后的Exo-miR-410-3p模拟物仍保持其外泌体生物学特性,可被HTR-8/Svneo细胞摄取,将miR-410-3p高效递送至HTR-8/Svneo细胞中。

既往研究认为TRAF6介导的p38 MAPK信号通路可能是miR-410-3p的靶点^[27],并受其调控^[56]。该通路参与炎症、凋亡、细胞分化和细胞周期调控^[57, 58],并在滋养层细胞蜕膜和分娩中发挥重要作用^[59]。研究表明,LRIP通过myd88/TRAF6/p38 MAPK通路抑制中性粒细胞的活化^[60]。此外,miR-146a通过TRAF6/p38 MAPK通路负调控促炎细胞因子的分泌^[61]。本研究进一步发现,JSP含药血清干预后,HTR-8/Svneo细胞中p-p38蛋白表达水平显著回升。结合细胞摄取实验中外泌体荧光强度降低的结果,提示JSP含药血清可能通过减少细胞对Exo-miR-410-3p的摄取,从而解除miR-410-3p对TRAF6/p38 MAPK通路的抑制。后续的功能干预实验也能够证实TRAF6是miR-410-3p下游的关键效应分子。

寿胎丸含药血清已被证明能促进滋养层细胞的增殖、侵袭和迁移^[62],并诱导母胎免疫耐受^[63]。寿胎丸与西药联合使用对预防复发性SA方面更为有效,且无副作用^[64]。JSP相比于寿胎丸,具有配伍严谨、药性平和的特点,在本研究中JSP含药血清逆转Exo-miR-410-3p对HTR-8/Svneo细胞EMT、迁移和侵袭的抑制作用,这与上述研究的结论相符。JSP还能通过抑制铁死亡有效缓解复发性SA^[65]。而在本研究动物实验中,JSP则通过阻止Exo-miR-410-3p到达母胎界面降低胚胎吸收率。

本研究存在若干局限性。第一,考虑到中药复方的复杂性,本研究未能完全解析其多成分-多靶点的作用机制。中药复方中的多种活性成分可能存在协同、拮抗或代谢干扰作用,且煎煮过程中的化学反应亦可能影响最终药效物质的含量。本研究药效学结果仅反映了整体的生物效应,而具体哪些化合物起主导作用、其体内药代动力学行为如何,尚需借助系统药理学、高通量筛选及体内外代谢组学等方法进行深入探究。此外,不同批次药材的质量控制标准亦有待进一步完善,以降低潜在的批次间变异对结论稳定性的影响。第二,本研究的体内实验每组动物样本量为5只,较小的样本量可能导致实验的可重复性差,未来需扩大样本量以验证结论的稳定性。

综上所述,本研究表明JSP可能通过抑制HTR-8/Svneo细胞对Exo-miR-410-3p的摄取,促进滋养细胞EMT、迁移和侵袭,并减少胚胎流产,这可能是JSP改善

自然流产的重要机制。本研究为深入理解外泌体 miR-410-3p 在母胎界面中的功能提供了新的视角。

Declaration of interests: The authors declare no competing interests.

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