

丝氨酸蛋白酶抑制剂 E1 过表达通过诱导 M2 型巨噬细胞极化促进三阴性乳腺癌细胞增殖与紫杉醇耐药

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摘要:目的 探讨丝氨酸蛋白酶抑制剂 E1 (SERPINE1) 对三阴性乳腺癌细胞肿瘤免疫微环境的影响及其与紫杉醇 (PTX) 耐药性的关系。方法 采用 0~40 $\mu\text{mol/L}$ 的 PTX 处理三阴性乳腺癌细胞系 MDA-MB-231, 并利用 CCK-8 法测定 PTX 对 MDA-MB-231 细胞的半数抑制浓度 (IC_{50} 值)。通过低剂量逐步递增的方法, 在体外建立 PTX 耐药模型 (MDA-MB-231/PTX)。分别将 SERPINE1 过表达质粒或 SERPINE1 siRNA 转染至野生型和耐药型 MDA-MB-231 细胞株, 以实现 SERPINE1 的过表达或敲低。采用 Western blotting 检测各组细胞中 SERPINE1 的表达水平, 以评估转染效率。利用 Hoechst 33258 染色法评估细胞凋亡情况, 并通过 Western blotting 测定凋亡相关的活化天冬氨酸特异性半胱氨酸蛋白酶 (cleaved-caspase 3) 的表达水平。采用 EdU 和 CCK-8 法检测细胞增殖活力。将各组乳腺癌细胞与巨噬细胞共培养后, 利用流式细胞术和 Western blotting 检测巨噬细胞的 M1、M2 极化水平, 并计算 M1/M2 值。进一步构建裸鼠皮下移植瘤模型, 通过监测肿瘤生长及免疫组化染色验证其体内作用。结果 SERPINE1 的过表达抑制 MDA-MB-231 细胞的凋亡并促进细胞增殖 ($P < 0.01$), 而敲低 SERPINE1 则促进 MDA-MB-231/PTX 细胞的凋亡并抑制细胞增殖 ($P < 0.01$)。此外, SERPINE1 高表达的乳腺癌细胞有助于巨噬细胞向 M2 型极化、抑制 M1 极化、降低 M1/M2 比值 ($P < 0.01$)。体内移植瘤实验同样证实, 过表达 SERPINE1 促进肿瘤生长 ($P < 0.01$)。结论 在 MDA-MB-231 三阴性乳腺癌细胞中, SERPINE1 的过表达促进细胞增殖、抑制细胞凋亡, 并增强对 PTX 的耐药性。SERPINE1 参与调控乳腺癌微环境中巨噬细胞的极化状态, 促进 M2 型巨噬细胞的极化。

关键词: 三阴性乳腺癌; 丝氨酸蛋白酶抑制剂 E1; 紫杉醇耐药; 肿瘤免疫微环境; M2 型巨噬细胞极化

SERPINE1 overexpression promotes proliferation and paclitaxel resistance of triple-negative breast cancer cells by inducing M2 macrophage polarization

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Abstract: Objective To investigate the regulatory effect of Serpin Family E Member 1 (SERPINE1) on immune microenvironment and paclitaxel (PTX) resistance of triple-negative breast cancer (TNBC) cells. **Methods** CCK-8 assay was used to determine the half-maximal inhibitory concentration of PTX in TNBC cell line MDA-MB-231. In wild-type MDA-MB-231 cells and a PTX-resistant MDA-MB-231 cell line (MDA-MB-231/PTX) established by stepwise increasing low-dose PTX treatment, the effects of Western blot-verified transfection with SERPINE1 overexpression plasmids or SERPINE1 siRNAs on cell apoptosis were evaluated using Hoechst 33258 staining and by detecting expression levels of cleaved caspase-3 using Western blotting. The changes in proliferation of the transfected cells were assessed using EdU and CCK-8 assays. The breast cancer cells with different treatments were co-cultured with macrophages, and M1 and M2 polarization of the macrophages were analyzed with flow cytometry and Western blotting. In nude mouse models bearing subcutaneous breast cancer cell xenografts, the effects of SERPINE1 overexpression and knockdown in the engrafted cells on tumor growth and PTX resistance were evaluated. **Results** SERPINE1 overexpression significantly inhibited apoptosis and promoted proliferation of MDA-MB-231 cells, and SERPINE1 knockdown obviously promoted apoptosis and inhibited proliferation of MDA-MB-231/PTX cells. The macrophages co-cultured with SERPINE1-overexpressing breast cancer cells showed enhanced M2 polarization and suppressed M1 polarization with a lowered M1/M2 ratio. In the tumor-bearing nude mouse models, SERPINE1 overexpression in the engrafted cells resulted in significantly accelerated tumor growth. **Conclusion** In MDA-MB-231 cells, SERPINE1 overexpression promotes cell proliferation, inhibits apoptosis, and enhances PTX resistance. SERPINE1 plays a regulatory role in macrophage polarization in the immune microenvironment of breast cancer, and its high expression promotes M2 polarization of the macrophages.

Keywords: triple-negative breast cancer; serpin family E member 1; paclitaxel resistance; tumor immune microenvironment; M2 macrophage polarization

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乳腺癌是全球女性最常见的恶性肿瘤类型之一。根据世界卫生组织 2022 年公布的数据, 乳腺癌占女性新诊断恶性肿瘤的 23.8%^[1]。根据肿瘤是否表达雌激素受体、孕激素受体以及人类表皮生长因子 2 (HER2), 乳腺癌被划分为 3 大核心亚型: 激素受体阳性/HerbB2 阴性

型(占比70%)、ErbB2阳性型(占比15%至20%)以及三阴性型(即3种标准肿瘤分子标志物均不表达,占比15%)。三阴性乳腺癌因其高度的异质性和侵袭性特征,以及治疗选项的有限性,其复发与转移的风险显著高于其他2种亚型^[2],导致在治疗手段的选择及治疗效果上均不如其他亚型乐观。因此,对其生物标志物的探索和新型治疗方法的研究成为当前亟待解决的科研问题。

研究表明,三阴性乳腺癌化疗耐药性的形成机制复杂多样,涉及肿瘤微环境、药物外排机制、癌症干细胞与大量肿瘤细胞的复杂交互,以及多条信号通路的异常调控^[3,4]。随着三阴性乳腺癌分子分型研究与基因组测序技术的不断进步,其靶向治疗领域取得了显著进展^[5],例如,SF1126和吉非替尼联合通过PI3K/AKT-mTOR通路诱导三阴性乳腺癌细胞凋亡^[6],卡铂连续靶向PARP可抑制三阴性乳腺癌的原发性肿瘤生长和远处转移^[7],然而,新靶向药物的长期安全性和患者的耐受性仍需临床试验中进行验证。标准治疗方案仍以化疗为主,而化疗耐药性问题成为治疗成功的主要障碍^[3]。紫杉醇(PTX),作为紫杉烷类药物的代表,广泛应用于各类恶性肿瘤的抗微管治疗^[8]。在三阴性乳腺癌治疗中,PTX扮演着核心角色,但其耐药性的出现严重制约了临床应用效果^[9],因此,亟需深入探究导致该类型乳腺癌化疗耐药的分子机制。

丝氨酸蛋白酶抑制剂家族E成员1(SERPINE1),亦称纤溶酶原激活因子抑制剂-1基因,归属于丝氨酸蛋白酶抑制剂超家族。SERPINE1蛋白主要作为组织型纤溶酶原激活因子与尿激酶型纤溶酶原激活因子的抑制物而发挥作用^[10]。研究发现,SERPINE1能增强癌细胞的转移与侵袭能力,同时抑制癌细胞凋亡,进而促进肿瘤的增殖与扩散^[11]。此外,SERPINE1还可能扮演着乳腺癌血管重塑过程中的关键调控角色^[12]。在多种癌症中,如乳腺癌^[13-15]、胃癌^[16]、卵巢癌^[17,18]、膀胱癌^[19]、口腔癌^[20]和结直肠癌^[21],组织或血液中SERPINE1的高表达均被视为患者预后不佳的重要标志。另有报道指出,SERPINE1蛋白在癌症的化疗耐药机制中发挥着关键作用^[22]。有文献通过体外研究发现,SERPINE1可通过调节血管生成因子血管内皮生长因子A(VEGFA)的表达,促进三阴性乳腺癌细胞对PTX产生耐药^[23]。肥胖三阴性乳腺癌患者中,SERPINE1是介导相关肿瘤放射耐药性的关键因素^[24]。然而,作为影响肿瘤微环境的关键因子,SERPINE1如何与微环境组分,如免疫细胞、肿瘤相关纤维母细胞及免疫调节分子等进行相互作用,进而影响乳腺癌的机制还不明晰^[25,26]。此外,有证据表明SERPINE1与肿瘤微环境的重塑存在关联^[27],但如何有效地将SERPINE1作为乳腺癌治疗的潜在靶标仍需进一步研究。因此,深入探究肿瘤免疫微环境在SERPINE1介导的PTX耐药机制中的作用显得尤为重要。

本研究通过构建体外PTX耐药细胞模型与体内移植瘤模型相结合的方法,系统性探讨SERPINE1对三阴性乳腺癌(TNBC)耐药特性、细胞增殖与凋亡,及其对肿瘤微环境中巨噬细胞向M2型极化进程的调控作用。本研究首次在乳腺癌中从体外和体内2个层面探索SERPINE1在癌细胞与巨噬细胞互作中的作用,可为未来以SERPINE1为靶点的治疗策略的研发,尤其是在减轻耐药性和提升治疗效果方面,提供重要的理论依据和实验数据支持。

1 材料和方法

1.1 实验材料

CCK-8试剂盒、EdU试剂盒、Hoechst细胞凋亡染色试剂盒、BCA蛋白测定试剂盒(上海碧云天生物技术有限公司),荧光显微镜(CX41,奥林巴斯),蛋白酶抑制剂混合物(北京索莱宝科技有限公司)。

1.2 细胞培养

MDA-MB-231细胞(苏州海星生物),接种于含10%胎牛血清、100 U/mL青霉素和100 μg/mL链霉素的DMEM培养液,于37℃、5% CO₂的培养箱内培养,选对数期生长期细胞进行实验。

1.3 实验动物及模型建立

选取12只6周龄健康雌性BALB/c裸鼠(广州铭格生物科技有限公司),所有动物实验操作均符合湘南学院动物实验伦理委员会的规定(伦理批号:2023DWLL064)。将裸鼠随机分为3组(4只/组):阴性对照(NC)组、SERPINE1敲低(SERPINE1^{KD})组和SERPINE1过表达(SERPINE1^{OE})组。取对数生长期的相应稳定转染细胞株(MDA-MB-231),调整细胞浓度后,将2.0×10⁶个细胞重悬于100 μL PBS中,注射至每只裸鼠的右侧乳腺脂肪垫内。自成瘤之日起,每隔7 d使用游标卡尺测量肿瘤的长径(L)和短径(W),并按公式 $V=(L \times W^2)/2$ 计算肿瘤体积,绘制生长曲线。饲养28 d后,将小鼠安乐死,完整剥离肿瘤组织并拍照。部分肿瘤组织于4%多聚甲醛中固定,用于后续HE及免疫组化染色分析。

1.4 CCK-8实验

将MDA-MB-231细胞(2×10⁵/孔)转移至12孔板中,在37℃、5% CO₂培养,当细胞汇合度达到80%以上时,用PBS冲洗2次,弃去上清液。用不同浓度(0、2、5、10、20、40 μmol/L)PTX处理MDA-MB-231细胞24 h,然后使用CCK-8试剂盒进行细胞增殖活力测定,具体操作方法参考产品说明书,最后计算不同条件处理下的IC₅₀。

1.5 MDA-MB-231/PTX耐药模型建立

通过对MDA-MB-231细胞进行6次PTX处理,获得PTX耐药细胞株(MDA-MB-231/PTX)^[28]。双目倒置显微镜下观察各时段细胞的生长情况。

1.6 细胞转染实验

选取对数期生长的MDA-MB-231细胞,分为3组:

空白对照组(Blank)、过表达阴性对照组(pcDNA4.0空载体,EV)、SERPINE1过表达组(pcDNA4.0-SERPINE1表达载体,OE)。选取对数期生长的MDA-MB-231/PTX细胞分为3组:空白对照组(Blank)、siRNA阴性对照组(NC)、SERPINE1敲低组(siRNA)。空白组细胞正常培养,其余4组依照Lipofectamine2000转染试剂方法将空载质粒(EV)、SERPINE1过表达质粒(OE)、NC-siRNA(NC)和SERPINE1-siRNA(siRNA)转染至各组细胞,培养48 h后进行后续研究。

1.7 Hoechst染色实验

收集各组细胞,PBS清洗2次,加入0.5 mL固定液,室温固定10 min,加入0.5 mL Hoechst33258染色液,染色5 min,然后用PBS洗涤2次。滴入抗荧光淬灭封片液,置于荧光显微镜($\times 400$)下观察凋亡细胞(蓝色荧光)并拍照。

1.8 EdU免疫荧光染色

将MDA-MB-231和MDA-MB-231/PTX细胞按照 1.5×10^4 /mL的密度接种于96孔板,以1:1000稀释的EdU培养2.5 h后,用4%多聚甲醛室温固定15 min,然后用含3% BSA的PBS清洗3次后,含0.3% Triton X-100的PBS室温孵育15 min,最后用EdU染料进行细胞核染色,在荧光显微镜下观察。

1.9 Western blotting检测

用预冷的PBS洗涤细胞,使用含有蛋白酶抑制剂混合物的RIPA裂解缓冲液裂解细胞提取蛋白,通过BCA蛋白测定试剂盒测定蛋白质浓度。总共加载45 μ g蛋白质提取物,通过10% SDS聚丙烯酰胺凝胶电泳分离,并转移到聚偏二氟乙烯(PVDF)膜上(Millipore)。在室温下在5%牛奶中的TBST中封闭1 h,将膜与一抗(ABCAM, SERPINE1: 1/1000, cleaved-caspase 3: 1/500, YMI1: 1/100, Arg-1: 1/4000, iNOS: 1/1000, GAPDH: 1/5000)在4 $^{\circ}$ C下孵育过夜,然后与HRP标记的绵羊抗兔二抗(ABCAM, 1:10 000)或HRP标记的绵羊抗小鼠二抗(ABCAM, 1:20 000)孵育1 h,然后加入化学发光试剂(TAKARA),通过化学发光检测系统检测蛋白质条带,并通过Image-Pro Plus 6.0分析蛋白条带的灰度值。

1.10 巨噬细胞共培养

人单核细胞系THP-1购自中科院细胞库。使用含10%胎牛血清的RPMI 1640完全培养基培养细胞。使用50 ng/mL佛波醇-12-肉豆蔻酸酯-13-乙酸酯(PMA, Sigma-Aldrich)刺激THP-1细胞48 h,获得M0型巨噬细胞。将其接种到Transwell基底外侧腔室中,培养48 h后,通过流式细胞术检查CD86、CD163阳性细胞的百分比。将转染处理的MDA-MB-231和MDA-MB-231/PTX细胞分别接种于Transwell上室,设立仅含DMEM完全培养基的孔作为空白对照。

1.11 HE染色

取石蜡包埋的肿瘤组织切片,经二甲苯脱蜡和梯度酒精水化后,采用苏木精-伊红进行常规染色。染色完成后,切片经梯度酒精脱水、二甲苯透明,最后以中性树脂封固。在光学显微镜下观察并采集各组肿瘤组织的病理学形态图像。

1.12 免疫组化

取石蜡包埋的肿瘤组织切片,经脱蜡水化后进行抗原修复。随后用3% H_2O_2 孵育以阻断内源性过氧化物酶活性,并用山羊血清封闭非特异性位点。切片分别与一抗(Arg-1: 1/5000, iNOS: 1/2000)4 $^{\circ}$ C孵育过夜,次日与辣根过氧化物酶(HRP)标记的二抗(ABCAM, 1:1000)室温孵育。采用DAB显色试剂盒进行显色,并用苏木精复染细胞核。最后经脱水、透明、封固后,于显微镜下观察并采集图像。

1.13 统计学分析

使用SPSS 21.0统计软件对数据进行分析,计量资料以均数 \pm 标准差表示,采用ANOVA检验结合事后Tukey's test检验进行组间比较。所有检验均为双侧检验, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 三阴性乳腺癌细胞中SERPINE1表达干预

Western blotting检测结果显示,过表达载体可上调MDA-MB-231细胞中SERPINE1的表达水平($P < 0.01$,图1A、B),而SERPINE1-siRNA可敲降MDA-MB-231/PTX细胞中SERPINE1的表达($P < 0.05$,图1C、D)。

2.2 SERPINE1对三阴性乳腺癌细胞增殖活力和细胞凋亡的影响

MDA-MB-231细胞经SERPINE1过表达处理后,CCK-8(图2A)和EdU染色(图2C)结果均显示细胞增殖活力增强($P < 0.01$)。MDA-MB-231/PTX细胞转染SERPINE1-siRNA后,细胞增殖活力则减弱($P < 0.01$,图2B、D)。Hoechst染色和Western blotting结果显示,MDA-MB-231细胞经SERPINE1过表达处理后,MDA-MB-231细胞损伤无显著改变,但Cleaved-caspase 3表达减弱($P < 0.01$,图3A、C、D)。而MDA-MB-231/PTX细胞经SERPINE1-siRNA处理后,细胞损伤增强,Cleaved-caspase 3表达上调($P < 0.01$,图3B、D)。

2.3 SERPINE1对三阴性乳腺癌细胞PTX耐药性的影响

CCK-8结果显示,MDA-MB-231细胞经SERPINE1过表达处理后,PTX的 IC_{50} 值上调($P < 0.01$,图4A),其对PTX耐药增强;相反,MDA-MB-231/PTX细胞经SERPINE1-siRNA处理后,PTX的 IC_{50} 值降低($P < 0.01$,图4B)。

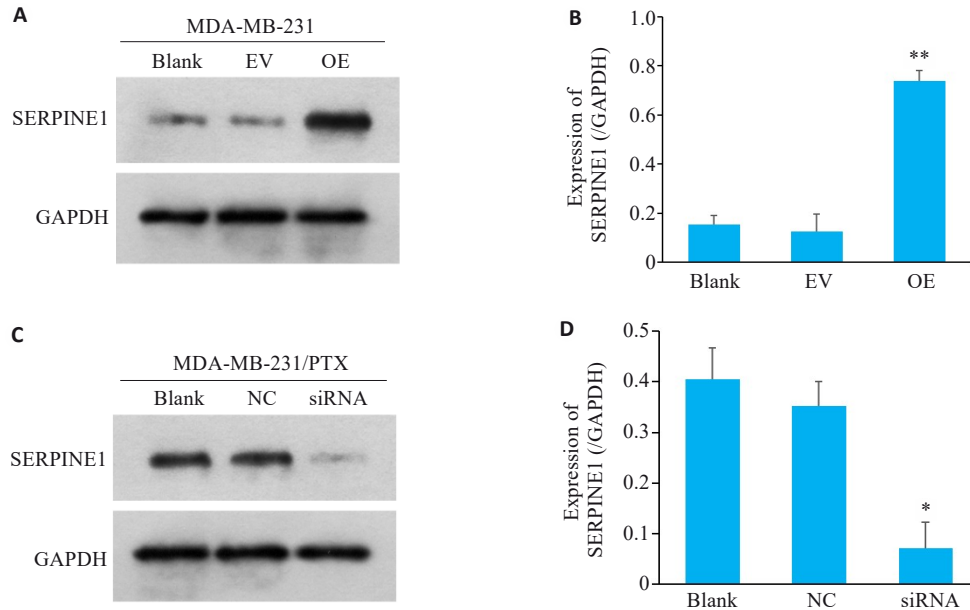


图1 MDA-MB-231细胞和MDA-MB-231/PTX细胞分别进行SERPINE1过表达和敲低干预
Fig.1 SERPINE1 overexpression in MDA-MB-231 cells and SERPINE1 knockdown in MDA-MB-231/PTX cells. **A, B:** Western blotting for detecting SERPINE1 protein expression in MDA-MB-231 cells transfected with pcDNA4.0-SERPINE1 and vector plasmid (** $P < 0.01$ vs EV group, $n = 3$); **C, D:** Western blotting for detecting SERPINE1 protein expression in MDA-MB-231/PTX cells transfected with SERPINE1-siRNA and NC-siRNA. GAPDH was used as the loading control (* $P < 0.05$ vs NC group, $n = 3$).

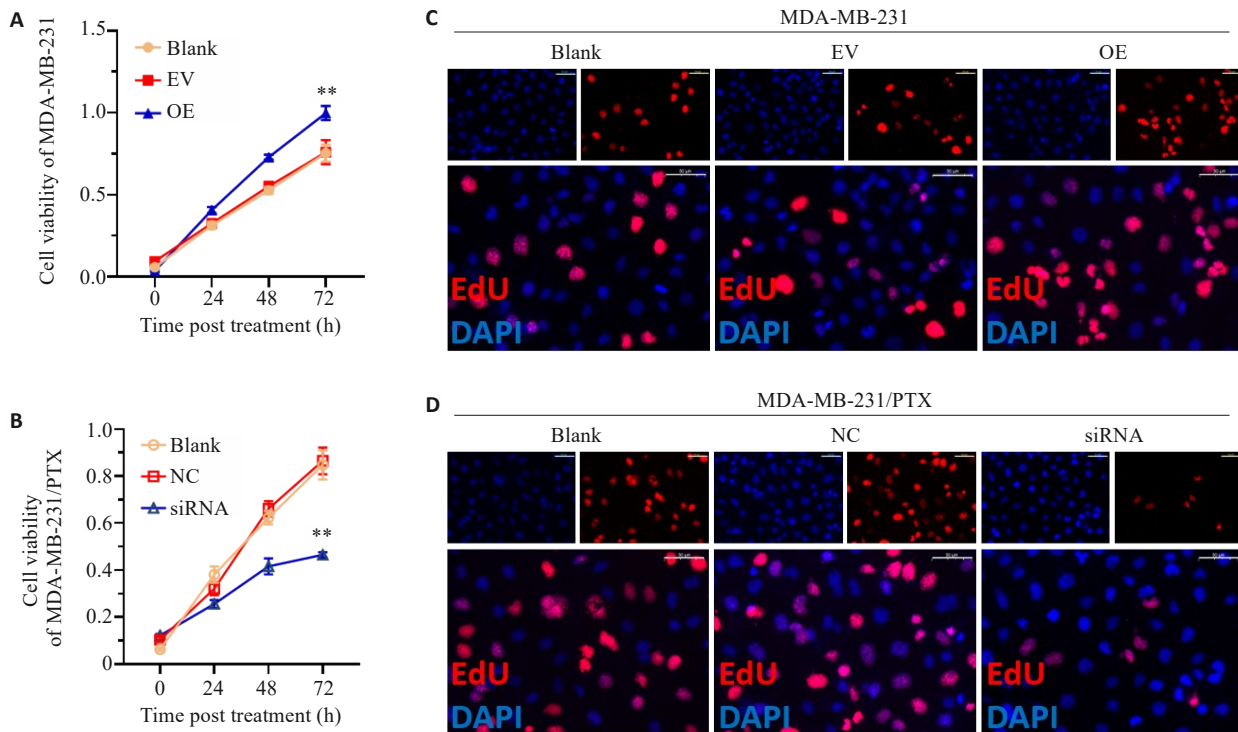


图2 SERPINE1对三阴性乳腺癌细胞增殖活力影响

Fig. 2 Proliferative activity of MDA-MB-231 cells with SERPINE1 overexpression and MDA-MB-231/PTX cells with SERPINE1 knockdown. **A, B:** Results of CCK-8 assay of MDA-MB-231 cells (**A**) transfected with pcDNA4.0-SERPINE1 or the vector plasmid and MDA-MB-231/PTX cells (**B**) transfected with SERPINE1-siRNA or NC-siRNA (** $P < 0.01$ vs EV group or NC group). **C, D:** EdU staining of MDA-MB-231 cells with SERPINE1 overexpression and MDA-MB-231/PTX cells with SERPINE1 knockdown (scale bar=50 μm).

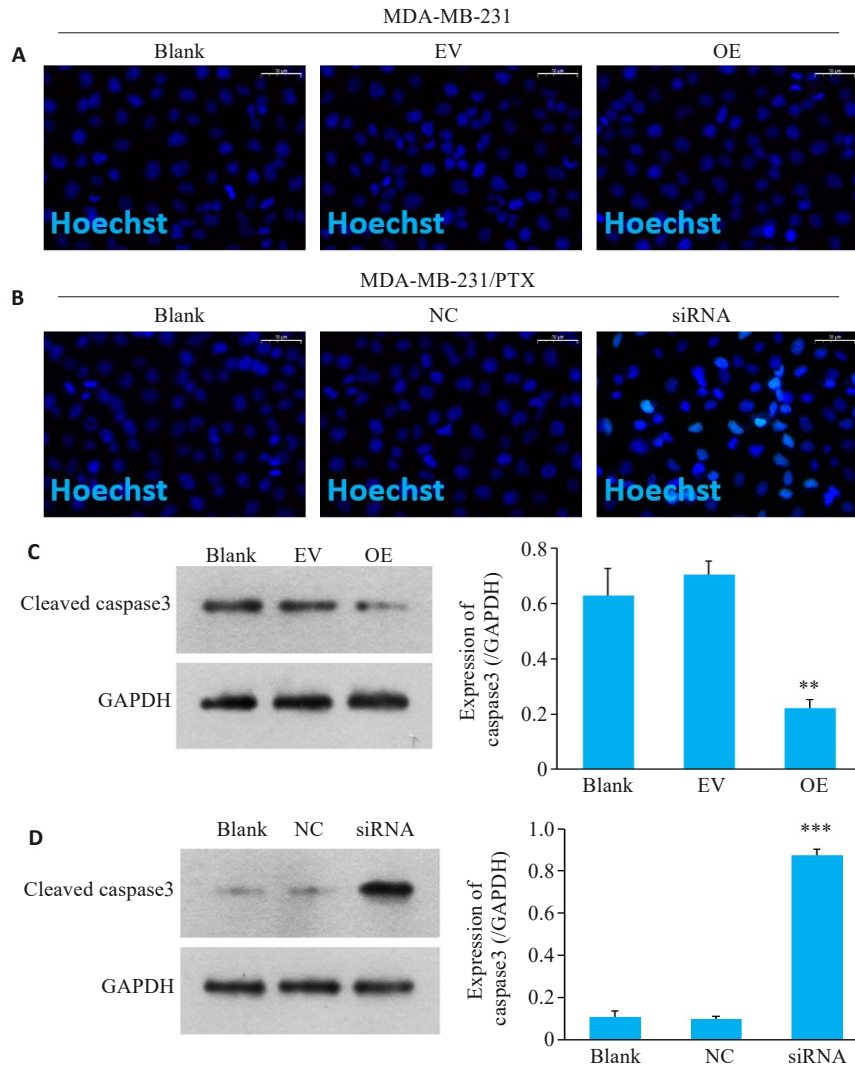


图3 SERPINE1对三阴性乳腺癌细胞凋亡影响

Fig.3 Changes in apoptosis of MDA-MB-231 cells with SERPINE1 overexpression and MDA-MB-231/PTX cells with SERPINE1 knockdown. **A, B:** DNA damage detected by Hoechst staining in MDA-MB-231 cells with SERPINE1 overexpression and in MDA-MB-231/PTX cells with SERPINE1 knockdown (scale bar=50 μ m). **C:** Western blotting for detecting protein levels of cleaved caspase-3 in MDA-MB-231 cells transfected with pcDNA4.0-SERPINE1 versus the vector plasmid (** P <0.01 vs EV group). **D:** Western blotting for detecting protein levels of cleaved caspase-3 in MDA-MB-231/PTX cells transfected with SERPINE1-siRNA versus NC-siRNA (** P <0.001 vs NC group, n =3).

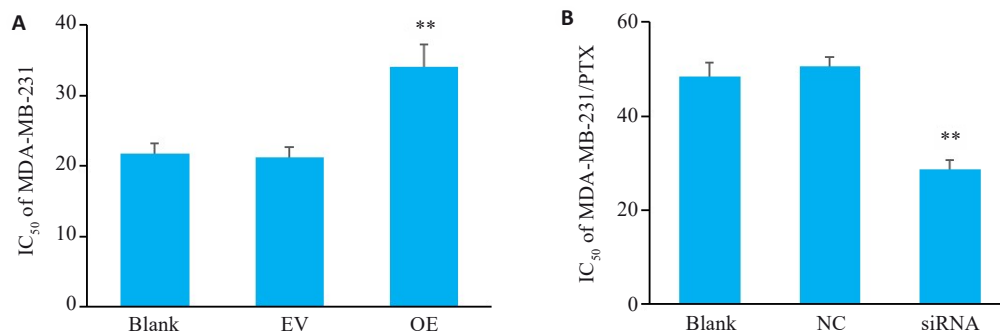


图4 SERPINE1对三阴性乳腺癌细胞PTX耐药性的影响

Fig.4 Changes in paclitaxel resistance of MDA-MB-231 cells with SERPINE1 overexpression and MDA-MB-231/PTX cells with SERPINE1 knockdown. **A:** CCK-8 assay for determining IC₅₀ value of paclitaxel in MDA-MB-231 cells transfected with pcDNA4.0-SERPINE1 versus the vector plasmid (** P <0.01 vs EV group). **B:** CCK-8 assay for determining the IC₅₀ value of paclitaxel in MDA-MB-231/PTX cells transfected with SERPINE1-siRNA versus NC-siRNA (** P <0.01 vs NC group, n =3).

2.4 三阴性乳腺癌细胞中SERPINE1对巨噬细胞M2极化影响

MDA-MB-231细胞经SERPINE1过表达处理后,可促进巨噬细胞M2的极化($P<0.01$,图5A,B),M2型巨

噬细胞标记蛋白YM1、Arg-1蛋白表达水平增强($P<0.01$,图5E,F)。敲低MDA-MB-231/PTX细胞SERPINE1表达后,巨噬细胞M2极化水平下调($P<0.05$,图5C,D),YM1、Arg-1蛋白表达水平减弱($P<0.01$,图5G,H)。

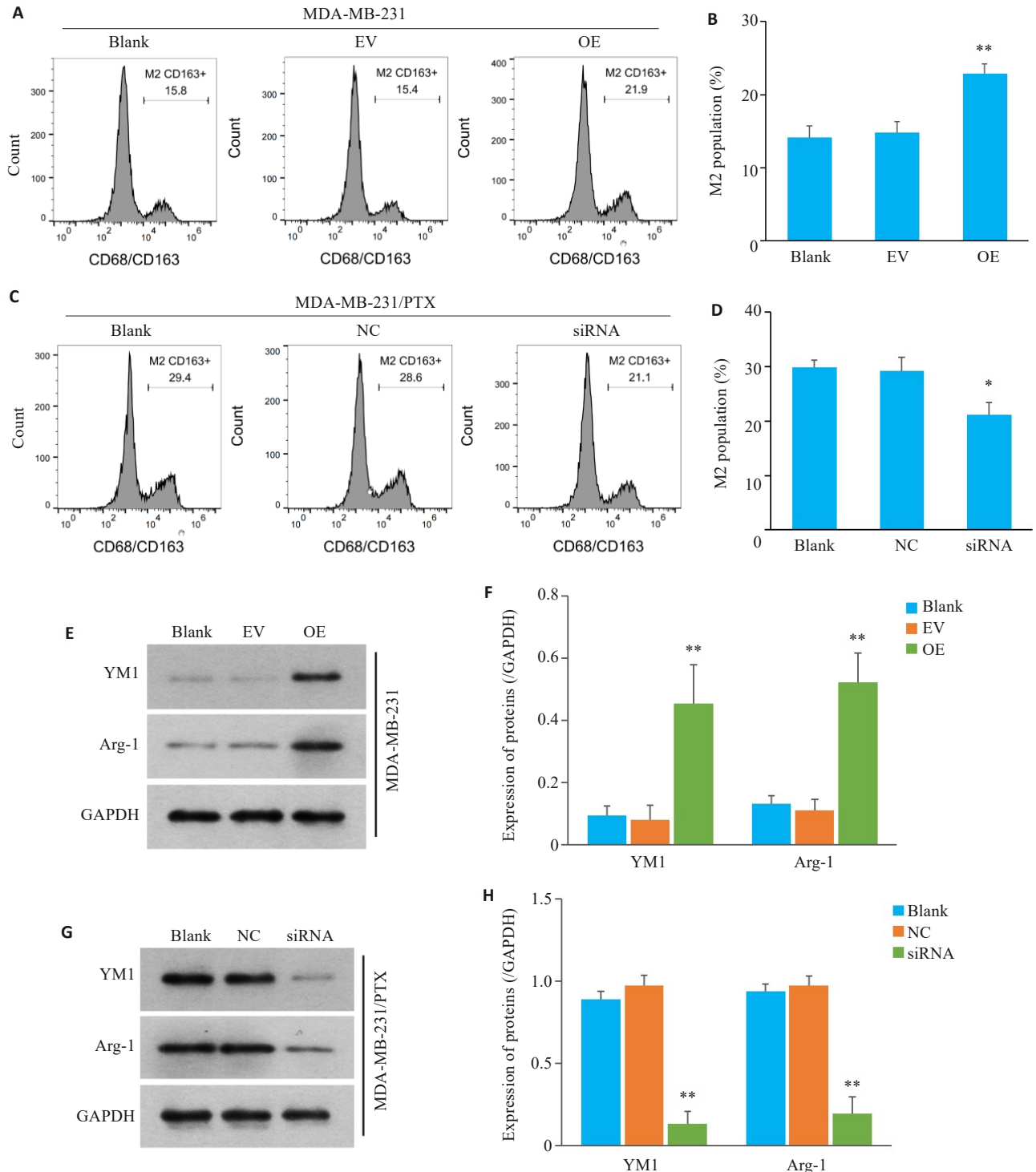


图5 三阴性乳腺癌细胞中SERPINE1对巨噬细胞M2极化影响

Fig.5 Effect of SERPINE1 overexpression in MDA-MB-231 cells and SERPINE1 knockdown in MDA-MB-231/PTX cells on M2 polarization of the co-cultured macrophages. **A, B**: Flow cytometry for analyzing the proportion of CD163⁺ macrophages co-cultured with SERPINE1-overexpressing MDA-MB-231 cells (** $P<0.01$ vs EV group). **C, D**: Flow cytometry for analyzing the proportion of CD163⁺ macrophages co-cultured with MDA-MB-231/PTX cells with SERPINE1 knockdown (* $P<0.05$ vs NC group). **E, F**: Western blotting for detecting the protein expression of YM1 and Arg-1 in the macrophages from the microenvironment of MDA-MB-231 cells with or without SERPINE1 overexpression (** $P<0.01$ vs EV group). **G, H**: Western blotting for detecting the protein expression of YM1 and Arg-1 in macrophages from the microenvironment of MDA-MB-231/PTX cells with or without SERPINE1 knockdown (** $P<0.01$ vs NC group, $n=3$).

2.5 三阴性乳腺癌细胞中SERPINE1对巨噬细胞M1极化影响

MDA-MB-231细胞经SERPINE1过表达处理后,共培养体系中M1型巨噬细胞(CD86⁺)的比例下降($P<0.05$,图6A,B),同时其功能标志物iNOS的蛋白表达量亦随之降低($P<0.01$,图6E,F)。反之,MDA-MB-231/PTX细胞经SERPINE1-siRNA处理后,共培养体系中M1型巨噬细胞的占比提升($P<0.01$,图6C,D),并伴有iNOS蛋白表达水平的同步上调($P<0.01$,图6G,H)。

有iNOS蛋白表达水平的同步上调($P<0.01$,图6G,H)。

2.6 三阴性乳腺癌细胞中SERPINE1对巨噬细胞M1/M2比值的影响

与过表达SERPINE1的MDA-MB-231细胞共培养后,巨噬细胞的M1/M2比值降低($P<0.01$,图7A)。反之,在与敲低SERPINE1的MDA-MB-231/PTX细胞共培养后,巨噬细胞的M1/M2比值增高($P<0.01$,图7B)。

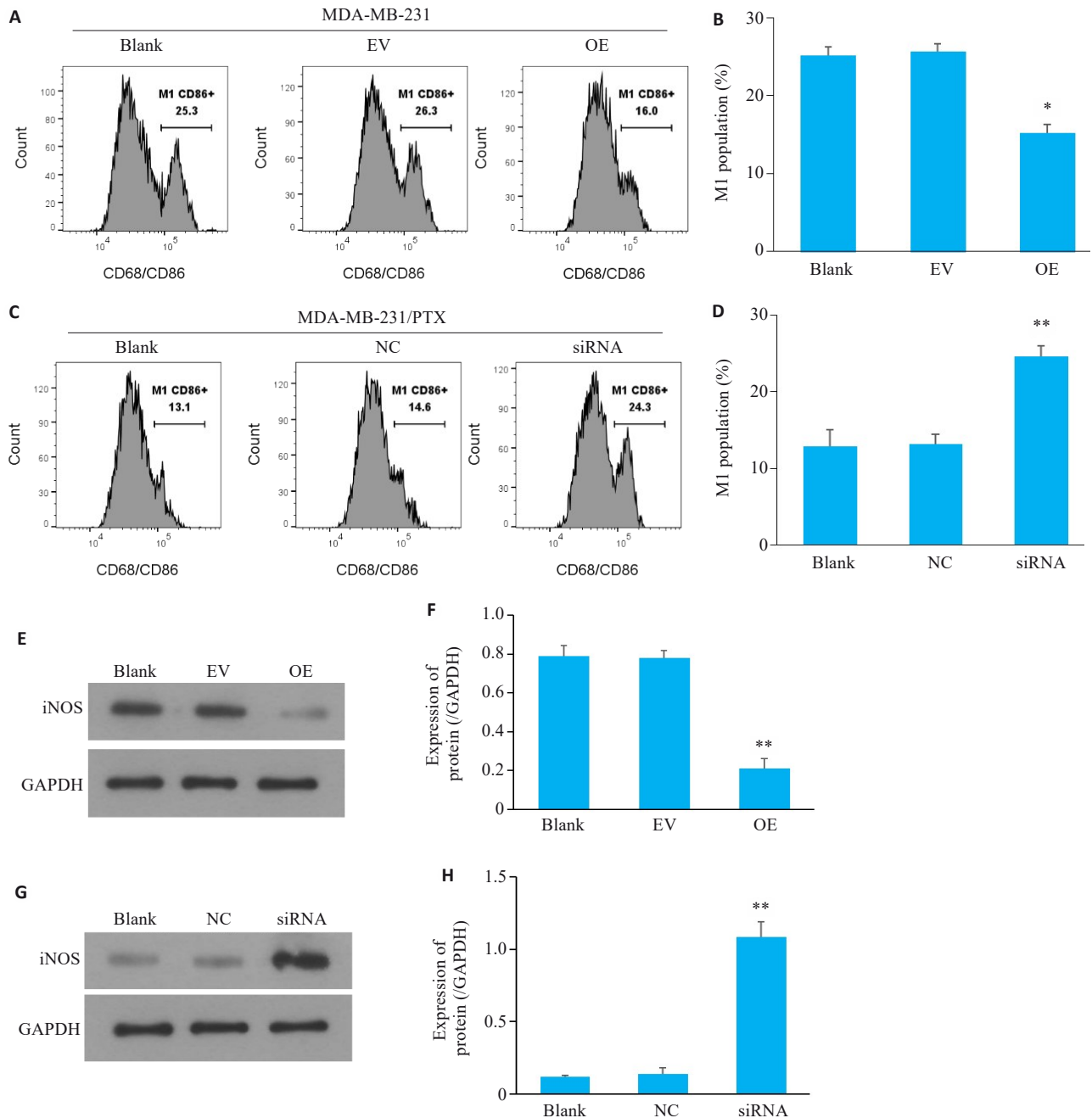


图6 三阴性乳腺癌细胞中SERPINE1对巨噬细胞M1极化影响

Fig.6 M1 polarization of the macrophages co-cultured with MDA-MB-231 cells with SERPINE1 overexpression or MDA-MB-231/PTX cells with SERPINE1 knockdown. A, B: Flow cytometry for analyzing the proportion of CD86⁺ macrophages co-cultured with MDA-MB-231 cells with SERPINE1 overexpression ($*P<0.05$ vs EV group). C, D: Flow cytometry for analyzing the proportion of CD86⁺ macrophages co-cultured with MDA-MB-231/PTX cells with SERPINE1 knockdown ($**P<0.01$ vs NC group). E, F: Western blotting for detecting the protein expression of iNOS in macrophages from the microenvironment of MDA-MB-231 cells with or without SERPINE1 overexpression ($**P<0.01$ vs EV group). G, H: Western blotting for detecting the protein expression of iNOS in macrophages from the microenvironment of MDA-MB-231/PTX cells with or without SERPINE1 knockdown ($**P<0.01$ vs NC group, $n=3$).

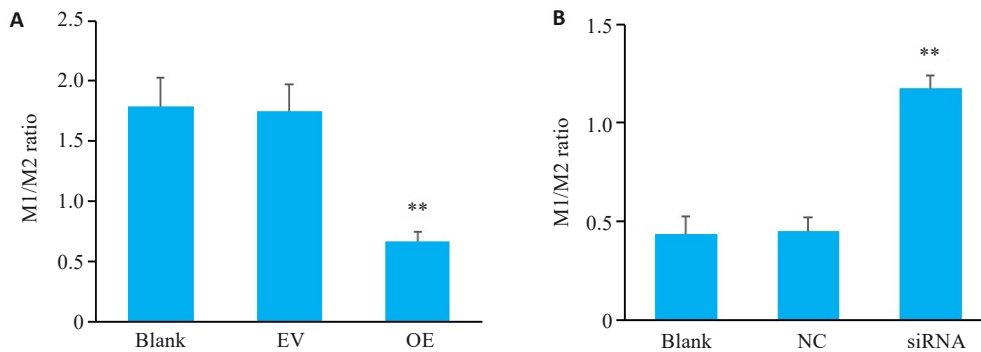


图7 三阴性乳腺癌细胞中SERPINE1对巨噬细胞M1/M2比值影响

Fig. 7 M1/M2 ratio of the macrophages co-cultured with MDA-MB-231 cells with SERPINE1 overexpression (A; ** $P < 0.01$ vs EV group) and MDA-MB-231/PTX cells with SERPINE1 knockdown (B; ** $P < 0.01$ vs blank group, $n=3$).

2.7 SERPINE1在体内调控三阴性乳腺癌生长及巨噬细胞极化

与NC组相比, SERPINE1^{OE}组的肿瘤体积增大、生长加快,而SERPINE1^{KD}组的肿瘤生长则受到抑制($P < 0.01$,图8A、B)。肿瘤组织中的蛋白表达水平验证了各组中SERPINE1的成功干预(图8C)。病理学分析显示,敲低SERPINE1可导致肿瘤组织出现大面积坏死(图8D)。免疫组化结果进一步揭示, SERPINE1过表达增强M2型巨噬细胞标志物Arg-1的表达,并抑制M1型标志物iNOS的表达;而在SERPINE1敲低组中,此效应发生逆转(图8E、F)。

3 讨论

TNBC作为一种高度恶性的亚型, TNBC治疗领域亟待攻克的一大难题^[29]。在此背景下,紫杉醇作为临床治疗TNBC的一线药物,获得性耐药成为TNBC治疗领域亟待攻克的一大难题^[30]。此外,从肿瘤微环境的角度出发,治疗TNBC的研究也取得了显著进展。研究表明,靶向肿瘤微环境成分与肿瘤靶向治疗相结合可减少肿瘤生长、转移和化疗耐药,例如,抑制整合素信号通路可以调节细胞外基质蛋白介导的乳腺癌细胞的耐药性^[31]。肿瘤相关巨噬细胞分泌的高水平IL-10可能通过调节肿瘤细胞中的抗凋亡信号通路来影响人乳腺癌细胞的紫杉醇耐药^[32]。然而, SERPINE1在肿瘤微环境中如何发挥作用目前仍有待深入研究。本课题旨在探讨SERPINE1在乳腺癌和巨噬细胞微环境中的作用,阐述SERPINE1可能与乳腺癌紫杉醇耐药的机制。

SERPINE1对肿瘤的恶性生物学特性具有显著的促进作用。研究揭示, SERPINE1的高表达与乳腺癌患者中增强的DNA修复能力密切相关,进而引发肿瘤细胞对放疗的抗性增强^[24]。在胃癌研究领域, SERPINE1被证实具有促进肿瘤细胞增殖及上皮-间质转化的双重作用^[33]。在我们之前的研究中, SERPINE1的敲除显著抑制了三阴性乳腺癌细胞存活并诱导了细胞凋亡。同

时, SERPINE1沉默导致血管生成关键因子血管内皮生长因子A的下调。此外,抑制SERPINE1减弱了体内乳腺癌肿瘤生长^[23]。因此, SERPINE1的高表达,可能与TNBC的耐药性、高转移特性密切相关。本研究结果进一步证明, MDA-MB-231乳腺癌细胞中过表达SERPINE1促进细胞增殖、抑制细胞凋亡,增强对PTX的耐药性。因此,本研究在前期研究的基础上,进一步阐明了SERPINE1促进三阴性乳腺癌耐药的作用机制。

肿瘤相关巨噬细胞在TNBC耐药机制中扮演着关键角色,因此,调节肿瘤微环境被视为一种具有潜力的TNBC治疗途径。研究指出, SERPINE1在肿瘤微环境中呈现高度聚集状态^[34]。在骨肉瘤的复发进程中, SERPINE1通过激活肿瘤相关纤维细胞,并进一步诱导巨噬细胞向M2型极化,同时促进肿瘤细胞发生上皮-间质转化,从而驱动骨肉瘤的转移^[35]。此外, TNBC细胞释放的SERPINE1能刺激肿瘤相关内皮细胞分泌CCL5,进而加剧TNBC的转移与增殖等恶性特征^[34]。本研究表明, TNBC中SERPINE1的过表达可促进M2型巨噬细胞的极化;相反,当TNBC中SERPINE1的表达受到干扰时, M2型极化的比例则明显降低。这表明SERPINE1可能通过调控肿瘤微环境中的巨噬细胞来参与TNBC的耐药机制。癌症相关成纤维细胞(CAF)与肿瘤相关巨噬细胞(TAM)作为肝细胞癌肿瘤微环境的两大核心成分,共同促进了肿瘤的进展。研究揭示, CAF通过分泌CXCL12来诱导巨噬细胞向M2型极化,并促进SERPINE1蛋白的分泌;同时发现TAMs分泌的SERPINE1蛋白能增强肝细胞癌细胞的恶性程度^[36]。这揭示了巨噬细胞M2型极化、肿瘤微环境与SERPINE1蛋白之间存在着错综复杂的相互作用。巨噬细胞分泌的SERPINE1蛋白是否也能反向影响TNBC细胞的PTX耐药性及恶性行为,值得在后续研究中深入探讨。综上所述,本研究提供了坚实的体外及体内实验证据,阐明SERPINE1是驱动TNBC耐药和恶性进展的关键分子。它通过直接作用于癌细胞和间接调控

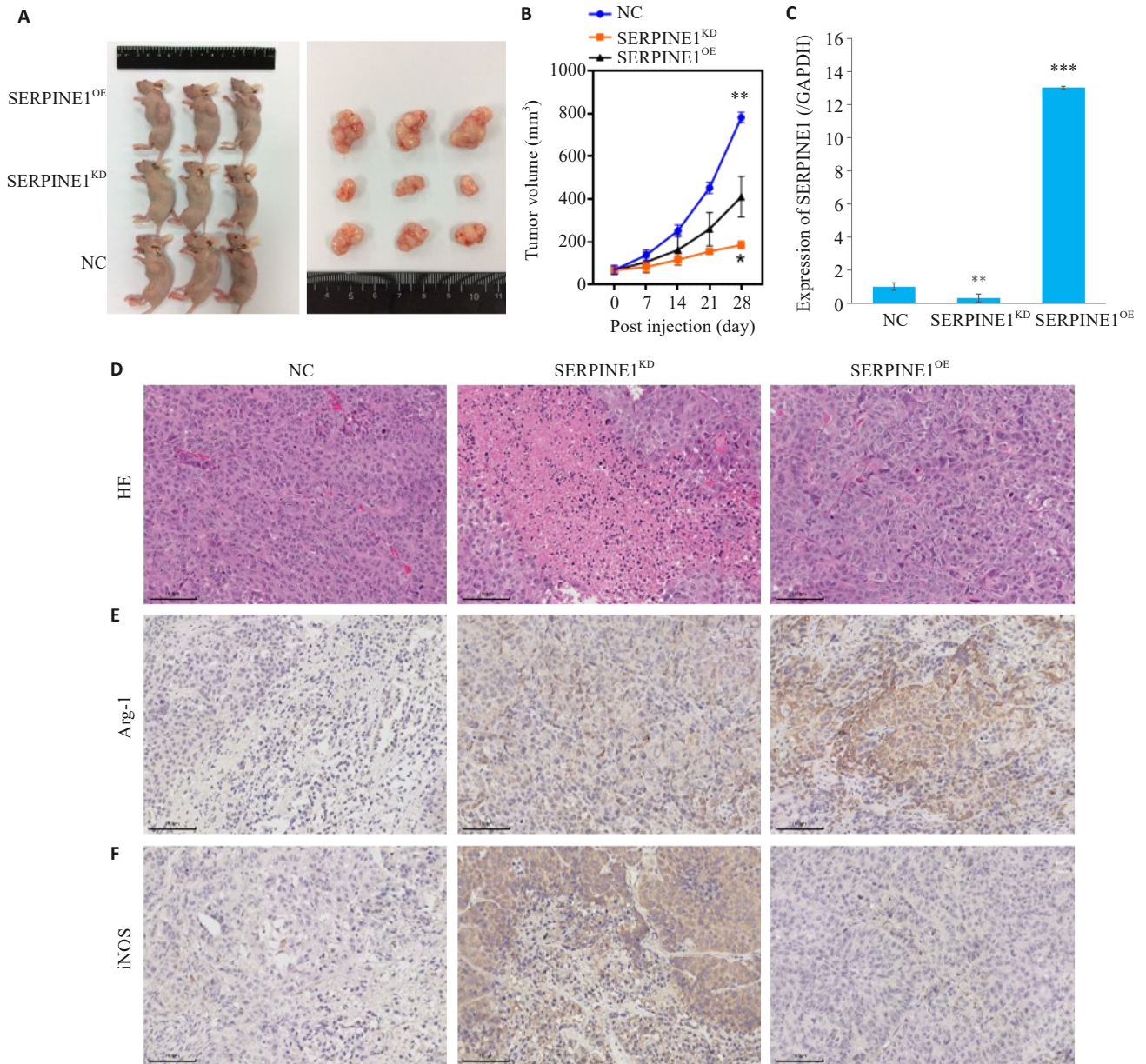


图8 SERPINE1在体内对三阴性乳腺癌生长的影响及对巨噬细胞极化的调控

Fig.8 Regulatory effect of SERPINE1 expression level on growth of triple-negative breast cancer xenografts and macrophage polarization in nude mice. **A:** Appearance of the tumor-bearing nude mice and the dissected tumors. **B:** Tumor growth curves over time in each group. **C:** Protein expression level of SERPINE1 in the xenograft tumor tissues from each group. **D:** HE staining of xenograft tumor tissues. **E:** Immunohistochemistry for the M2 macrophage marker Arg-1 in xenograft tumor tissues. **F:** Immunohistochemistry for the M1 macrophage marker iNOS in xenograft tumor tissues (scale bar=100 μm). **P*<0.05, ***P*<0.01, ****P*<0.001 vs NC group. *n*=6.

免疫微环境两条通路发挥作用,提示SERPINE1有望成为攻克TNBC耐药难题、提升治疗效果的有效新靶点。

综上所述,本研究通过体外及体内实验证实了SERPINE1在TNBC中扮演着关键的促癌角色:它通过促进细胞增殖、抑制凋亡及诱导M2型巨噬细胞极化,共同加剧了肿瘤的恶性进展与PTX耐药。这一发现为理解TNBC耐药机制提供了新的视角,并为靶向SERPINE1的治疗策略奠定了坚实的理论基础。本研究仍有一些问题有待深入探索。例如,需在更广泛的TNBC细胞模型中验证本研究结论的普适性,深入阐明其调控巨噬细胞极化的具体分子通路,均有待进一步探

索。未来研究方向将聚焦于推动SERPINE1靶向治疗,从而为其从基础研究走向临床应用提供关键支持。

Declaration of interests: The authors declare no competing interests.

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