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KLK5过表达对裸鼠皮下移植瘤生长及顺铂敏感性的影响

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[摘 要] **目的：**探讨激肽释放酶5（KLK5）过表达对宫颈癌细胞的增殖、侵袭及顺铂（DDP）敏感性的影响，并阐明其作用机制。**方法：**采用Western blotting法验证KLK5稳定转染过表达的宫颈癌细胞（ME180-OE-KLK5）。取对数生长期的宫颈癌ME180-NC-KLK5和OE-KLK5细胞，分别将其接种于裸鼠皮下建立皮下移植瘤模型，造模成功后裸鼠随机分为生理盐水对照组（NC-KLK5+0.9%NaCl组）、DDP治疗组（NC-KLK5+DDP组）、KLK5过表达组（OE-KLK5+0.9%NaCl组）和KLK5过表达联合DDP组（OE-KLK5+DDP组），每组5只。NC-KLK5+DDP组和OE-KLK5+DDP组裸鼠按照5 mg·kg^{-1}的比例腹腔注射DDP；NC-KLK5+0.9%NaCl组和OE-KLK5+0.9%NaCl组裸鼠按照0.01 mL·g^{-1}的比例腹腔注射生理盐水。每2 d称裸鼠质量，并记录瘤体的长径和短径，计算肿瘤体积，绘制瘤体生长曲线，于第14天给药结束后24 h，处死裸鼠，剥离瘤体并称质量。采用HE染色法观察各组裸鼠肿瘤组织病理形态表现，免疫组织化学染色法观察各组裸鼠肿瘤组织中KLK5、Ki67和基质金属蛋白酶9（MMP-9）蛋白表达水平。**结果：**与ME180-NC-KLK5细胞比较，ME180-OE-KLK5细胞中KLK5蛋白表达水平升高（$P<0.05$）。皮下移植瘤种植后第1周，各组裸鼠进食和活动状态良好，体质量逐渐增长。第2周开始进入给药阶段，NC-KLK5+0.9%NaCl组裸鼠进食和活动状态及体质量较第1周无明显变化；与NC-KLK5+0.9%NaCl组比较，NC-KLK5+DDP组裸鼠开始出现食欲减退，体质量不增长，活动状态减弱；第3周药物治疗期间，NC-KLK5+0.9%NaCl组裸鼠进食及活动状态较第2周无明显变化，开始出现体质量不增长；与NC-KLK5+0.9%NaCl组比较，NC-KLK5+DDP组裸鼠进食和活动状态明显减弱，体质量降低。与NC-KLK5+0.9%NaCl组比较，NC-KLK5+DDP组裸鼠移植瘤体积减小（$P<0.01$）；与NC-KLK5+DDP组比较，OE-KLK5+DDP组裸鼠移植瘤体积明显增大（$P<0.001$）；与NC-KLK5+0.9%NaCl组比较，OE-KLK5+0.9%NaCl组裸鼠移植瘤体积增大（$P<0.001$）；与OE-KLK5+0.9%NaCl组比较，OE-KLK5+DDP组裸鼠移植瘤体积差异无统计学意义（$P>0.05$）。与NC-KLK5+0.9%NaCl组比较，NC-KLK5+DDP组裸鼠移植瘤质量降低（$P<0.05$）；与NC-KLK5+DDP组比较，OE-KLK5+DDP组裸鼠瘤质量明显升高（$P<0.001$）；与NC-KLK5+0.9%NaCl组比较，OE-KLK5+0.9%NaCl组裸鼠瘤质量升高（$P<0.001$）；与OE-KLK5+0.9%NaCl组比较，OE-KLK5+DDP组裸鼠瘤体质量差异无统计学意义（$P>0.05$）。与NC-KLK5+0.9%NaCl组比较，OE-KLK5+0.9%NaCl组裸鼠移植瘤细胞核异质性更强；OE-KLK5+DDP组和NC-KLK5+DDP组裸鼠移植瘤细胞出现形态学改变，表现为细胞核固缩、碎裂，肿瘤细胞体积缩小以及出现坏死和凋亡等。与NC-KLK5+DDP组比较，OE-KLK5+DDP组裸鼠移植瘤坏死程度更明显。与NC-KLK5+0.9%NaCl组比较，NC-KLK5+DDP组裸鼠移植瘤组织中KLK5、Ki67和MMP-9蛋白表达水平降低（$P<0.05$）；与NC-KLK5+DDP组比较，OE-KLK5+DDP组裸鼠移植

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瘤组织中KLK5、Ki67和MMP-9蛋白表达水平升高（$P<0.001$）；与NC-KLK5＋0.9%NaCl组比较，OE-KLK5＋0.9%NaCl组裸鼠移植瘤组织中KLK5、Ki67和MMP-9蛋白表达水平升高（$P<0.001$）；与OE-KLK5＋0.9%NaCl组比较，OE-KLK5＋DDP组裸鼠移植瘤组织中KLK5、Ki67和MMP-9蛋白表达水平差异无统计学意义（$P>0.05$）。**结论**：KLK5过表达可促进DDP处理的宫颈癌ME180细胞裸鼠皮下移植瘤的生长，上调移植瘤组织中Ki-67和MMP-9蛋白表达，降低移植瘤对DDP的敏感性。

[关键词] 激肽释放酶5；宫颈肿瘤；顺铂；基质金属蛋白酶9；ME180细胞；移植瘤
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Effects of KLK5 overexpression on growth of subcutaneous xenograft tumor and cisplatin sensitivity in nude mice

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ABSTRACT **Objective**: To discuss the effects of kallikrein 5 (KLK5) overexpression on the proliferation, invasion and cisplatin (DDP) sensitivity of cervical cancer cells, and to clarify its mechanism. **Methods**: Western blotting method was used to verify the stable transfection and overexpression of KLK5 in the cervical cancer cell (ME180-OE-KLK5). The cervical cancer ME180-NC-KLK5 and ME180-OE-KLK5 cells in logarithmic growth phase were subcutaneously inoculated into the nude mice to establish the subcutaneous xenograft models. After successful modeling, the mice were randomly divided into normal saline control group (NC-KLK5＋0.9% NaCl group), DDP treatment group (NC-KLK5＋DDP group), KLK5 overexpression group (OE-KLK5＋0.9% NaCl group) and KLK5 overexpression combined with DDP group (OE-KLK5＋DDP group), with 5 mice in each group. The nude mice in NC-KLK5＋DDP group and OE-KLK5＋DDP group were given intraperitoneal injection of DDP at a dose of 5 $mg\cdot kg^{-1}$; the nude mice in NC-KLK5＋0.9% NaCl group and OE-KLK5＋0.9% NaCl group were given intraperitoneal injection of normal saline at a dose of 0.01 $mL\cdot g^{-1}$. The body weights of nude mice were measured every 2 d, and the long diameter and short diameter of the tumors were recorded to calculate the tumor volume and plot the tumor growth curve. At 24 h after the last administration on day 14, the nude mice were sacrificed, and the tumors were dissected and weighed. HE staining method was used to observe the pathomorphology of tumor tissue in the nude mice in various groups; immunohistochemistry staining method was used to observe the expression levels of KLK5, Ki67 and matrix metalloproteinase-9 (MMP-9) proteins in the tumor tissues of the nude mice in various groups. **Results**: Compared with ME180-NC-KLK5 cells, the expression level of KLK5 protein in ME180-OE-KLK5 cells was increased ($P<0.05$). In the first week after subcutaneous xenograft inoculation, the nude mice in various groups showed good feeding and activity status, and their body weights gradually increased. The drug administration phase started from the second week. During the drug treatment period, the feeding and activity status as well as body weight of the nude mice in NC-KLK5＋0.9%NaCl group showed no significant changes compared with the first week; compared with NC-KLK5＋0.9%NaCl group, the nude mice in NC-KLK5＋DDP group began to show loss of appetite, no increase in body weight, and decreased activity. During the drug treatment period in the third week, the feeding and activity status of the nude mice in NC-KLK5＋0.9%NaCl group showed no significant changes compared with the second week, while they began to show no increase in body weight; compared with NC-KLK5＋0.9%NaCl group, the feeding and activity status of the nude mice in

NC-KLK5+DDP group were significantly weakened, and their body weights decreased. Compared with NC-KLK5+0.9%NaCl group, the volume of xenograft tumor in NC-KLK5+DDP group was decreased ($P<0.01$); compared with NC-KLK5+DDP group, the volume of xenograft tumor OE-KLK5+DDP group was significantly increased ($P<0.001$); compared with NC-KLK5+0.9%NaCl group, the volume of xenograft tumor of the nude mice in OE-KLK5+0.9%NaCl group was increased ($P<0.001$); compared with OE-KLK5+0.9%NaCl group, the volume of xenograft tumors in the nude mice in OE-KLK5+DDP group showed no statistically significant difference ($P>0.05$). Compared with NC-KLK5+0.9%NaCl group, the weight of xenograft tumor of the nude mice in NC-KLK5+DDP group was decreased ($P<0.05$); compared with NC-KLK5+DDP group, the weight of xenograft tumors of the nude mice in OE-KLK5+DDP group was significantly increased ($P<0.001$); compared with NC-KLK5+0.9%NaCl group, the weight of xenograft tumor of the nude mice in OE-KLK5+0.9%NaCl group was increased ($P<0.001$); compared with OE-KLK5+0.9%NaCl group, the weight of xenograft tumors of the nude mice in OE-KLK5+DDP group showed no statistically significant difference ($P>0.05$). Compared with NC-KLK5+0.9%NaCl group, the xenograft tumor cells of the nude mice in OE-KLK5+0.9%NaCl group showed greater nuclear heterogeneity; the xenograft tumor cells of the nude mice in OE-KLK5+DDP group and NC-KLK5+DDP group showed cytomorphological changes, manifested as nuclear pyknosis and fragmentation, reduced cell volume, and the appearance of necrosis and apoptosis. Compared with NC-KLK5+DDP group, the degree of necrosis in xenograft tumor of the nude mice in OE-KLK5+DDP group was more pronounced. Compared with NC-KLK5+0.9%NaCl group, the expression levels of KLK5, Ki67 and MMP-9 proteins in xenograft tumor tissue of the nude mice in NC-KLK5+DDP group were decreased ($P<0.05$); compared with NC-KLK5+DDP group, the expression levels of KLK5, Ki67, and MMP-9 proteins in xenograft tumor tissue of the nude mice in OE-KLK5+DDP group were increased ($P<0.001$); compared with NC-KLK5+0.9%NaCl group, the expression levels of KLK5, Ki67 and MMP-9 proteins in xenograft tumor tissue of the nude mice in OE-KLK5+0.9%NaCl group were increased ($P<0.001$); compared with OE-KLK5+0.9%NaCl group, the expression levels of KLK5, Ki67 and MMP-9 in xenograft tumor tissue of the nude mice in OE-KLK5+DDP group showed no statistically significant differences ($P>0.05$). **Conclusion**: KLK5 overexpression can promote the growth of subcutaneous xenograft tumors of cervical cancer ME180 cells treated with DDP, up-regulate the expressions of Ki67 and MMP-9 in the xenograft tumor tissue, and reduce the sensitivity of the xenograft tumor to DDP.

KEYWORDS Kallikrein 5; Uterine cervical neoplasm; Cisplatin; Matrix metalloproteinase-9; ME180 cells; Xenograft tumor

宫颈癌是全球女性第4大常见癌症，其发病率和死亡率在女性恶性肿瘤中均位居前列[1]。化疗是宫颈癌系统性治疗的核心手段，在宫颈癌的治疗中具有不可替代的地位。顺铂（cisplatin，DDP）是宫颈癌的一线化疗药物，广泛应用于宫颈癌的同步放化疗、辅助治疗以及姑息治疗。化疗耐药性是宫颈癌临床治疗效果不佳的主要原因，降低了患者有效生存时间[2-3]。激肽释放酶（kallikreins，KLK）家族由15个高度同源的分泌型丝氨酸蛋白酶基因组成[4]，其中KLK5的异常表达和功能失活与癌症的发生发展及预后有密切关联。KLK5在不同肿瘤中的表达水平及其功能存在明显差异，其在基底细胞样乳腺癌[5]和高级别浆液性卵巢癌[6]中表达明显上调，而在前列腺癌[7]和阴道癌[8]中则下调或表现出潜在抑制作用，提示其功能具有组织环境依赖性。KLK5在肿瘤中的作用具有组织特异性和肿瘤类型特异性，有望作为癌症诊断的生物标志物和潜在治疗靶点[9-12]。已有研究[13]证实KLK5增强了宫颈癌放疗的辐射抗性，但对于KLK5在宫颈癌化疗中的作用机制及相关影响因素的研究较少，尚无明确结论。本研究探讨KLK5过表达对宫颈癌细胞增殖、侵袭和DDP敏感性的影响，通过建立宫颈癌OE-KLK5细胞皮下移植瘤裸鼠模型，观察KLK5过表达对皮下移植瘤生长及化疗敏感性的影响，为

宫颈癌的临床治疗提供新思路。

1 材料与方法

1.1 实验动物、主要试剂和仪器 雌性BALB/c裸鼠20只，体质量15～17 g，购自辽宁长生生物技术股份有限公司，实验动物生产许可证号：SCXK（辽）2020-0001。宫颈癌ME180-NC-KLK5细胞购自上海富恒生物科技有限公司，本课题组前期研究已基于此细胞系建立稳定转染KLK5过表达宫颈癌细胞（ME180-OE-KLK5）。DMEM基础培养基购自美国Hyclone公司，DDP购自山东思科捷生物技术有限公司，高效细胞裂解液购自上海碧云天生物科技有限公司，KLK5抗体购自英国Abcam公司，Ki67抗体购自杭州荟丹生物科技有限公司，基质金属蛋白酶9（matrix metalloproteinase 9，MMP-9）抗体、β-actin抗体和山羊抗兔二抗抗体购自武汉三鹰生物技术有限公司，HE染色试剂盒和免疫组织化学试剂盒购自北京索莱宝科技有限公司。倒置显微镜购自日本Olympus公司，Western blotting电泳仪和转膜仪购自上海天能公司，化学发光成像系统购自北京京仪集团有限责任公司。

1.2 细胞培养 ME180细胞培养于含10%胎牛血清和1%青-链霉素的DMEM培养基中，于37 ℃、5% CO_2培养箱中培养。待细胞密度达到80%～90%时，按1∶3或1∶4比例进行传代。

1.3 Western blotting法检测宫颈癌ME180细胞中KLK5蛋白表达水平 弃去培养皿旧培养基，沿侧壁缓慢加入磷酸盐缓冲液（phosphate buffered saline，PBS）洗涤细胞2次。收集细胞至离心管中，在4 ℃离心机中5 000 r·min^{-1}，离心5 min，弃去上清液，PBS缓冲液重悬细胞后，再次离心，弃去上清液。向离心管中加入适量细胞裂解液，冰上裂解30 min，每隔5 min涡旋混匀1次。4 ℃离心机中12 000 r·min^{-1}，离心15 min，收集上清液。预留部分上清液用于浓度测定，按照蛋白上清液∶蛋白上样缓冲液＝4∶1的比例加入5×蛋白上样缓冲液，沸水中煮10 min，分装后－20 ℃保存。采用BCA法测定蛋白浓度。电泳电压设置为150 V，时间50 min，90 V转膜90 min。5%脱脂牛奶封闭PVDF膜，室温摇床1 h。封闭后采用含吐温20的Tris缓冲盐溶液（Tris-Buffered Saline with Tween® 20，TBST）洗涤3次，每次10 min。加入KLK5抗体（1∶2 000）和β-actin抗体（1∶5 000）分别在4 ℃冰箱摇床孵育过夜。TBST洗涤3次，每次10 min。加入山羊抗兔二抗抗体（1∶2 000），室温摇床孵育1 h。TBST洗涤3次，每次10 min。在化学发光成像系统中显影成像。采用Image J软件分析蛋白条带灰度值，计算目的蛋白表达水平。目的蛋白表达水平＝目的蛋白条带灰度值/β-actin蛋白条带灰度值。

1.4 裸鼠皮下移植瘤制备和实验分组 取对数生长期的宫颈癌ME180-NC-KLK5和ME180-OE-KLK5细胞，使用PBS缓冲液调整细胞悬液密度为2×10^7 mL^{-1}，于裸鼠右侧腋窝背部皮下注射，细胞悬液注射体积为每只裸鼠100 μL。接种7 d后，若在皮下触诊发现质地坚硬且边缘清晰可辨的结节，可确认为移植瘤模型构建成功。当皮下移植瘤平均体积超过75 mm^{3}时，将裸鼠随机分为生理盐水对照组（NC-KLK5＋0.9%NaCl组）、DDP治疗组（NC-KLK5＋DDP组）、KLK5过表达组（OE-KLK5＋0.9%NaCl组）和KLK5过表达联合DDP组（OE-KLK5＋DDP组），每组5只。NC-KLK5＋0.9%NaCl组和NC＋KLK5＋DDP治疗组用于评估DDP对宫颈癌的疗效；OE-KLK5＋0.9%NaCl组和OE-KLK5＋DDP组用于评估KLK5过表达对DDP疗效的影响。NC-KLK5＋0.9%NaCl组和OE-KLK5＋0.9%NaCl组小鼠按照0.01 mL·g^{-1}腹腔注射生理盐水，每只约0.2 mL，持续2周，每周2次，共计4次。NC-KLK5＋DDP治疗组和OE-KLK5＋DDP组小鼠按照5 mg·kg^{-1}的比例腹腔注射DDP，每只0.2 mL，持续2周，每周2次，共计4次。于第1、5、9和14天分别给药，每2 d记录1次裸鼠的进食和活动状态、体质量及肿瘤最长径和最短径，计算各组裸鼠移植瘤体积。肿瘤体积＝0.5×肿瘤最长径×肿瘤最短径^2。治疗结束后24 h将小鼠脱颈处死，采用75%乙醇消毒后剥离出瘤体组织，生理盐水清洗后擦干，并对瘤体称质量。

1.5 HE染色观察各组移植瘤组织病理形态表现 收集裸鼠瘤体，置于4%多聚甲醛中固定24 h后，进行梯度乙醇脱水、二甲苯透明、浸蜡和石蜡包埋后制成切片。石蜡切片经过脱蜡水化后，采用HE染色试剂盒进行染色，二甲苯透明并封片，于显微镜下观察并采集图像。

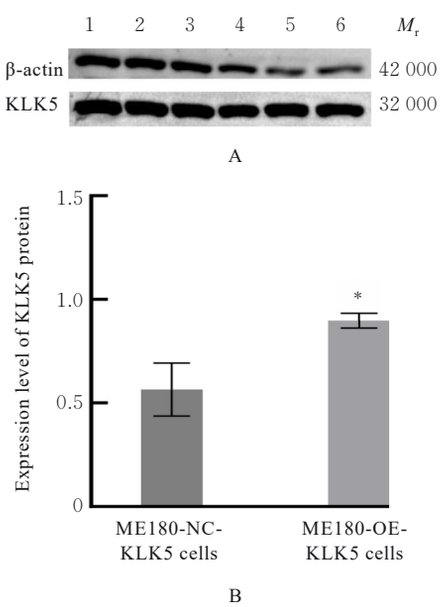
1.6 免疫组织化学染色检测各组移植瘤组织中KLK5、Ki67和MMP-9蛋白表达水平 石蜡切片进行脱蜡水化、抗原修复和非特定点封闭后，分别进行3种抗体孵育，DAB显色、复染和封片后，于显微

镜下观察并采集图像。采用Image J软件分析KLK5、Ki67和MMP-9蛋白表达情况，以平均光密度值代表目的蛋白表达水平。

1.7 统计学分析 采用Graphpad Prism 10.1统计软件进行统计学分析。细胞中KLK5蛋白表达水平，各组裸鼠体质量、皮下移植瘤体积和肿瘤组织中KLK5、Ki67及MMP9蛋白表达水平均符合正态分布且方差齐，以$\bar{x}\pm s$表示。多组间样本均数比较采用单因素方差分析，2组间样本均数比较采用SNK-q检验。以$P<0.05$为差异有统计学意义。

2 结 果

2.1 宫颈癌ME180细胞中KLK5蛋白表达水平 与ME180-NC-KLK5细胞比较，ME180-OE-KLK5细胞中KLK5蛋白表达水平升高（$P<0.05$）。见图1。



Lane 1－3：ME180-OE-KLK5 cells；Lane 4－6：ME180-NC-KLK5 cells. *$P<0.05$ compared with ME180-NC-KLK5 cells.

图1 Western blotting法检测宫颈癌细胞中KLK5蛋白表达电泳图(A)和直条图(B)

Fig. 1 Electrophoregram (A) and histogram (B) of expression of KLK5 protein in cerical cancer cells detected by Western blotting method

2.2 各组裸鼠体质量和一般情况 皮下移植瘤种植后第1周，各组裸鼠进食和活动状态良好，体质量逐渐增长。第2周开始进入给药阶段，药物治疗期间NC-KLK5＋0.9%NaCl组裸鼠进食和活动状态及体质量较第1周无明显变化；与NC-KLK5＋0.9%NaCl组比较，NC-KLK5＋DDP组裸鼠开始出现食欲减退，体质量不增，活动状态减弱。第3周药物治疗期间，NC-KLK5＋0.9%NaCl组裸鼠进食及活动状态较第2周无明显变化，开始出现体质量不增长；与NC-KLK5＋0.9%NaCl组比较，NC-KLK5＋DDP组裸鼠进食减退，活动状态减弱，体质量降低。见图2。

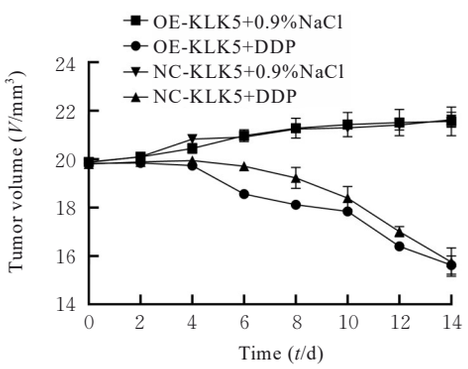


图2 各组裸鼠体质量折线图

Fig. 2 Line graphs of body weights of nude mice in various groups

2.3 各组裸鼠皮下移植瘤大体表现和体积 治疗结束后处死裸鼠并剥离瘤体，拍照记录，计算肿瘤体积并绘制瘤体生长折线图。结果显示：与NC-KLK5＋0.9%NaCl组比较，NC-KLK5＋DDP组移植瘤体积明显减小（$P<0.01$）；与NC-KLK5＋DDP组比较，OE-KLK5＋DDP组移植瘤体积明显增大（$P<0.001$）；与NC-KLK5＋0.9%NaCl组比较，OE-KLK5＋0.9%NaCl组裸鼠移植瘤体积增大（$P<0.001$）；与OE-KLK5＋0.9%NaCl组比较，OE-KLK5＋DDP组裸鼠移植瘤体积无明显变化，差异无统计学意义（$P>0.05$）。见图3和4。

2.4 各组裸鼠移植瘤质量 与NC-KLK5＋0.9%NaCl组比较，NC-KLK5＋DDP组裸鼠移植瘤质量降低（$P<0.05$）；与NC-KLK5＋DDP组比较，OE-KLK5＋DDP组裸鼠瘤质量明显降低（$P<0.001$）；与NC-KLK5＋0.9%NaCl组比较，OE-KLK5＋0.9%NaCl组裸鼠瘤质量升高（$P<0.001$）；与OE-KLK5＋0.9%NaCl组比较，OE-KLK5＋DDP组裸鼠瘤质量差异无统计学意义（$P>0.05$）。见图5。

2.5 各组裸鼠皮下移植瘤组织病理形态表现 与NC-KLK5＋0.9%NaCl组比较，OE-KLK5＋0.9%NaCl组裸鼠移植瘤细胞核异质性更强；OE-

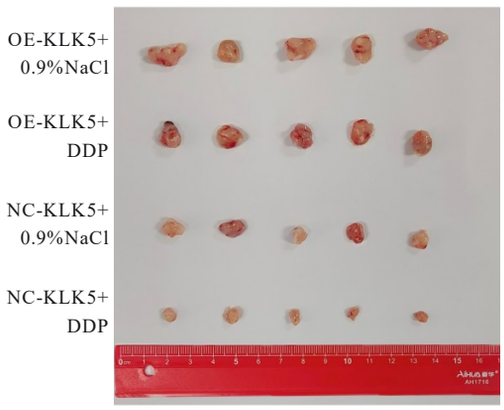
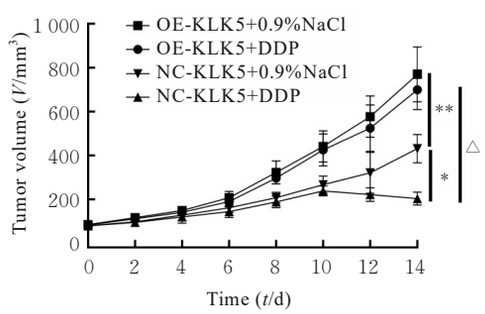


图3 各组裸鼠移植瘤大体表现

Fig. 3 General morphology of subcutaneous xenograft tumor of nude mice in various groups



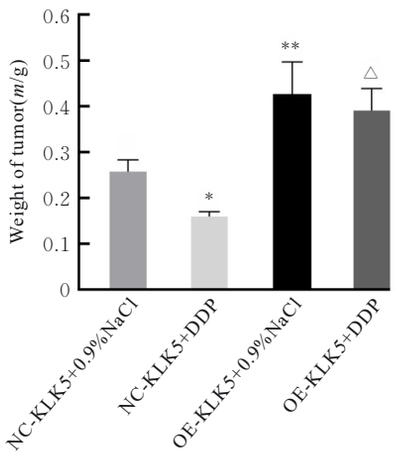
*$P<0.01$，**$P<0.001$ compared with NC-KLK5+0.9%NaCl group；△$P<0.001$ compared with NC-KLK5+DDP group.

图4 不同时间点各组裸鼠移植瘤体积

Fig. 4 Volumes of subcutaneous xenograft tumor of nude mice in various groups at different time points

KLK5+DDP组和NC-KLK5+DDP组裸鼠移植瘤细胞出现细胞形态学改变，表现为细胞核固缩、碎裂，肿瘤细胞体积缩小以及出现坏死和凋亡等；与NC-KLK5+DDP组比较，OE-KLK5+DDP组裸鼠移植瘤坏死程度更明显。见图6。

2.6 各组裸鼠移植瘤组织中KLK5、Ki67和MMP-9蛋白表达水平 KLK5阳性染色表达定位于细胞质，呈现棕黄色或褐色颗粒时为阳性表达；Ki67阳性染色表达定位于细胞核，出现棕黄色或褐色颗粒时为阳性表达；MMP-9阳性染色表达定位于细胞膜和细胞质，当出现棕黄色或褐色颗粒时为阳性表达。各组裸鼠肿瘤组织中KLK5、Ki67和MMP-9蛋白表达趋势基本一致。与NC-KLK5+0.9%NaCl组比较，NC-KLK5+DDP组裸鼠移植瘤组织中KLK5、Ki67和MMP-9蛋白表达水平降低（$P<0.05$）；与NC-KLK5+DDP组比较，OE-KLK5+DDP组裸鼠移植瘤组织中KLK5、Ki67和MMP-9蛋白表达水平升高（$P<0.001$）；与NC-KLK5+0.9%NaCl组比较，OE-KLK5+0.9%NaCl组裸鼠移植瘤组织中KLK5、Ki67和MMP-9表达水平升高（$P<0.001$）；与OE-KLK5+0.9%NaCl组比较，OE-KLK5+DDP组裸鼠移植瘤组织中KLK5、Ki67和MMP-9蛋白表达水平差异无统计学意义（$P>0.05$）。见图7～12。



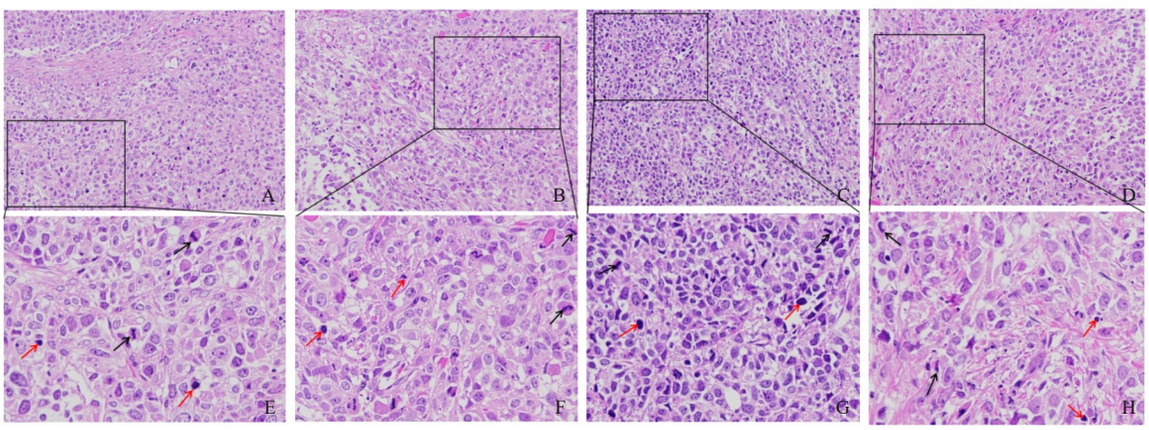
*$P<0.05$，**$P<0.001$ compared with NC-KLK5+0.9%NaCl group；△$P<0.001$ compared with NC-KLK5+DDP group.

图5 各组裸鼠皮下移植瘤质量

Fig. 5 Weights of subcutaneous xenograft tumor of nude mice in various groups

3 讨 论

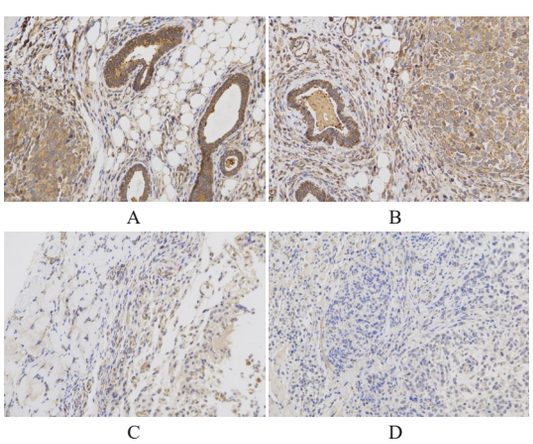
宫颈癌是全球女性主要死亡原因之一，放化疗是晚期宫颈癌的重要治疗手段，部分患者预后不良。研究[14]表明：KLK5在女性生殖系统恶性肿瘤中表达上调，且与肿瘤的进展和不良预后有关。在子宫内膜癌中，KLK5过表达患者的临床病理类型侵袭性更强，是预后的不良因素。KLK5过表达与卵巢癌患者不良临床结局有关联，且患者血清中KLK5浓度升高，提示KLK5可作为卵巢癌的潜在生物标志物，用于卵巢癌的早期诊断和预后评估[6, 15]。SUK等[16]分析165例接受根治性放疗的宫颈癌患者，发现KLK5过表达患者发病年龄更小、主动脉旁淋巴结更易受累、5年局部无复发生存率和无远处转移生存率更低，从而证实了KLK5过表达与宫颈癌的侵袭性有关。ZHOU等[13]发现KLK5过表达与放疗后的不良预后有关联，并通过



A,E:OE-KLK5+0.9%NaCl group; B,F:OE-KLK5+DDP group; C,G:NC-KLK5+0.9%NaCl group; D,H:NC-KLK5+DDP group. A-D:×200; E-H:×400. Red arrows represented nuclear pyknosis; black arrows represented nuclear fragmentation.

图 6　各组裸鼠肿瘤组织病理形态表现(HE)

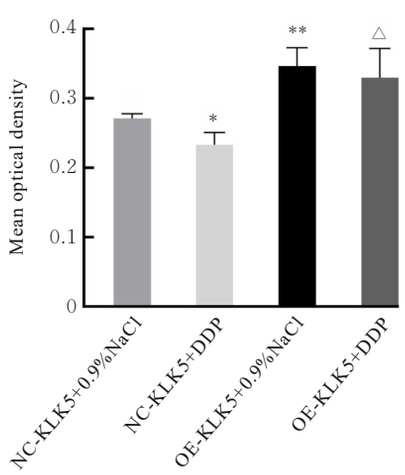
Fig. 6　Pathomorphology of tumor tissue of nude mice in various groups (HE)



A:OE-KLK5+0.9%NaCl group; B:OE-KLK5+DDP group; C:NC-KLK5+0.9%NaCl group; D:NC-KLK5+DDP group.

图 7　各组裸鼠肿瘤组织中 KLK5 蛋白表达情况(免疫组织化学,×200)

Fig. 7　Expressions of KLK5 protein in tumor tissue of nude mice in various groups (Immunohistochemistry, ×200)



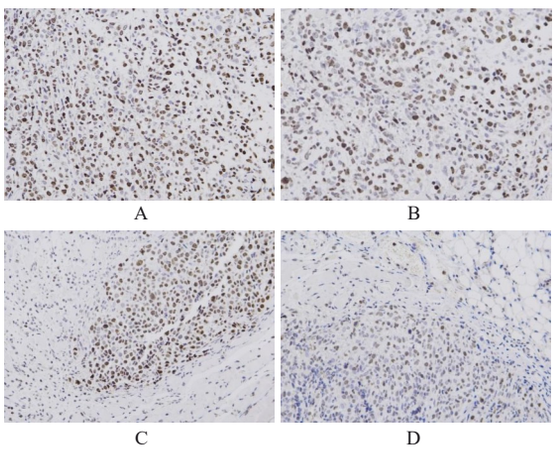
*$P<0.05$, **$P<0.001$ compared with NC-KLK5+0.9%NaCl group; △$P<0.001$ compared with NC-KLK5+DDP group.

图 8　各组裸鼠肿瘤组织中 KLK5 蛋白表达水平

Fig. 8　Expression levels of KLK5 protein in tumor tissue of nude mice in various groups

体内和体外实验证实了 KLK5 过表达可增加辐射抗性，而下调 KLK5 表达可增加放疗敏感性。

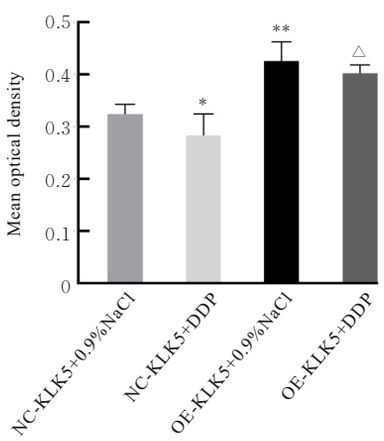
本研究结果显示：OE-KLK5+0.9%NaCl 组裸鼠皮下移植瘤体积和重量均大于 NC-KLK5+0.9%NaCl 组，表明 KLK5 过表达能促进宫颈癌细胞在裸鼠体内的生长。此外，OE-KLK5+0.9%NaCl 组裸鼠肿瘤组织中 Ki67 和 MMP-9 表达水平高于 NC-KLK5+0.9%NaCl 组。Ki67 的表达贯穿细胞增殖的全过程，通过极其复杂的方式与遗传物质及其他蛋白质结合，是核糖体合成的必需因子，其表达水平可反映细胞的增殖活跃程度，是评估细胞增殖的标志物。Ki67 表达水平越高，提示组织异质性越高，进而导致病情转归恶化[17-19]。MMP 是锌依赖性蛋白水解酶，对细胞外基质(extracellular matrix，ECM）成分有降解作用，而细胞和 ECM 成分间的相互作用对癌症进展至关重要[20]。MMP-9 是 MMP 家族的重要成员，其在多种恶性肿瘤中过表达，是宫颈癌独立的不良预后因素[21]。MMP-9 通过促进 ECM 蛋白降解以调节



A：OE-KLK5＋0.9%NaCl group；B：OE-KLK5＋DDP group；C：NC-KLK5＋0.9%NaCl group；D：NC-KLK5＋DDP group.

图9 各组裸鼠肿瘤组织中Ki67蛋白表达情况(免疫组织化学，×200)

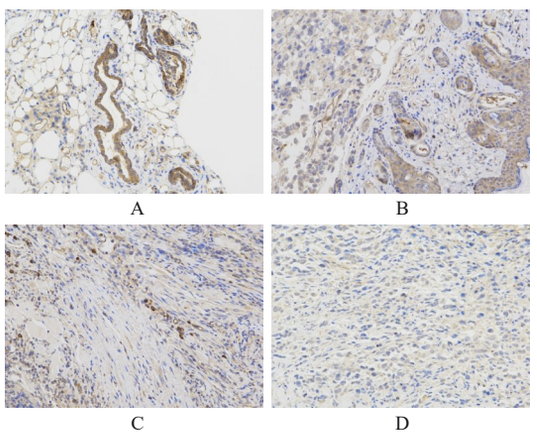
Fig. 9 Expressions of Ki67 protein in tumor tissue of nude mice in various groups (Immunohistochemistry, ×200)



*P<0.05，**P<0.001 compared with NC-KLK5＋0.9%NaCl group；△P<0.001 compared with NC-KLK5＋DDP.

图10 各组裸鼠肿瘤组织中Ki67蛋白表达水平

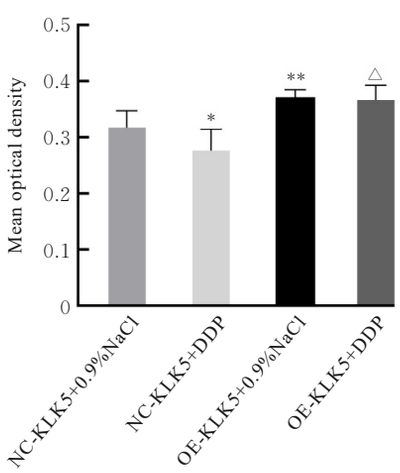
Fig. 10 Expression levels of Ki67 protein in tumor tissue of nude mice in various groups



A：OE-KLK5＋0.9%NaCl group；B：OE-KLK5＋DDP group；C：NC-KLK5＋0.9%NaCl group；D：NC-KLK5＋DDP group.

图11 各组裸鼠肿瘤组织中MMP-9蛋白表达情况(免疫组织化学，×200)

Fig. 11 Expressions of MMP-9 protein in tumor tissue of nude mice in various groups (Immunohistochemistry, ×200)



*P<0.05，**P<0.001 compared with NC-KLK5＋0.9%NaCl group；△P<0.001 compared with NC-KLK5＋DDP group.

图12 各组裸鼠肿瘤组织中MMP-9蛋白表达水平

Fig. 12 Expression levels of MMP-9 protein in tumor tissue of nude mice in various groups

ECM重构，使细胞和基底膜相连的Ⅳ型胶原纤维破裂，促进肿瘤细胞发生上皮-间充质转化(epithelial-mesenchymal transition，EMT)，增强肿瘤细胞侵袭及转移能力[22]。本研究通过免疫组织化学染色法检测各组肿瘤组织中Ki67和MMP-9蛋白表达水平，结果证实KLK5过表达上调了与宫颈癌增殖和侵袭相关蛋白Ki67及MMP-9表达，证明KLK5过表达增强宫颈癌细胞的增殖和侵袭能力。

本研究结果显示：OE-KLK5＋DDP组裸鼠移植瘤体积和质量与OE-KLK5＋0.9%NaCl组差异不明显，而NC-KLK5＋DDP组裸鼠移植瘤体积和质量小于NC-KLK5＋0.9%NaCl组。此外，OE-KLK5＋0.9%NaCl组裸鼠肿瘤组织中Ki67和MMP-9表达水平仍高于NC-KLK5＋0.9%NaCl，表明KLK5过表达可能通过维持较高的Ki67和MMP-9表达来降低移植瘤对DDP的敏感性，表明KLK5过表达可

能削弱DDP的化疗效果，导致化疗对肿瘤的生长抑制作用减弱。

本研究通过建立宫颈癌OE-KLK5细胞皮下移植瘤裸鼠模型，探讨KLK5过表达对皮下移植瘤生长及化疗敏感性的影响，结果显示：KLK5过表达能促进宫颈癌裸鼠皮下移植瘤的生长，并降低移植瘤对DDP的敏感性，同时上调与宫颈癌增殖和侵袭相关蛋白Ki67及MMP-9的表达，证实KLK5过表达促进了经DDP给药的宫颈癌裸鼠皮下移植瘤的生长，同时抑制了化疗诱导的凋亡坏死，降低了宫颈癌裸鼠皮下移植瘤的化疗敏感性，为宫颈癌的临床治疗提供了新思路。KLK5可能作为宫颈癌诊断和预后评估的潜在标志物，预测宫颈癌患者对化疗的敏感性，以期在将来的临床治疗中指导KLK5不同表达情况的宫颈癌患者进行化疗的必要性及DDP用药选择方案，从而改善患者的生存率和生存质量。

利益冲突声明：

所有作者声明不存在利益冲突。

作者贡献声明：

闫荣免参与实验操作和论文撰写，孙新婷参与实验实施和论文修改，关欣和程谕参与数据整理及分析，韩丽英参与论文审阅。

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