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# 干性基因Nanog在膀胱癌组织中的表达及其对膀胱癌细胞增殖与耐药的作用 王志,张艺,黄亚琴,等

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Title: Expression of Nanog in bladder cancer and its role in proliferation and drug resistance of bladder cancer cells

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摘要: 目的 探讨胚胎干细胞关键转录因子Nanog在膀胱癌组织中的表达及在膀胱癌5637细胞中的作用。 方法 采用免疫组化方法检测Nanog蛋白在46例膀胱癌组织中的表达。应用慢病毒载体上调5637细胞中Nanog的表达, 通过克隆形成实验检测细胞增殖, 通过MTT法检测细胞对化疗药物顺铂的敏感性。 结果 膀胱癌组织中Nanog的阳性率为50%, 且其表达与组织的病理分级呈正相关, 相关系数为0.989 ( $P<0.05$ ) ; Real-time PCR和Western blot结果表明感染慢病毒LV-Nanog的5637细胞株中Nanog mRNA和蛋白的表达明显高于感染慢病毒LV-Con的对照组细胞株。5637-C和5637-Nanog的克隆形成率分别为 $(4.6\pm0.9)\%$ 和 $(9.0\pm1.0)\%$ , 显示过表达Nanog的5637细胞克隆形成率上升 ( $P<0.01$ ) 。以5、10、20、40  $\mu\text{mol/L}$  顺铂处理细胞72 h, 对照组5637-C的存活率分别为 $(64.5\pm4.9)\%$ 、 $(53.1\pm4.6)\%$ 、 $(43.4\pm3.9)\%$ 和 $(21.0\pm2.7)\%$ , 5637-Nanog的存活率分别为 $(80.9\pm5.6)\%$ 、 $(68.5\pm4.2)\%$ 、 $(47.9\pm5.1)\%$ 和 $(25.2\pm4.2)\%$ , 在浓度为5  $\mu\text{mol/L}$ 和10  $\mu\text{mol/L}$ 时5637-Nanog对顺铂的敏感性降低。 结论 Nanog的表达增高与膀胱癌病理分级相关, Nanog基因的表达升高能增强膀胱癌细胞的增殖能力, 降低对顺铂化疗药物的敏感性。

Abstract: Objective To determine the expression of embryonic stem cells key transcription factor Nanog in bladder cancer tissues and its role in human bladder cancer cell line 5637. Methods Immunohistochemical assay were performed to detect the expression of Nanog gene in 46 tissue samples of bladder cancer. Nanog was over-expressed through lentivirus vector LV-Nanog in 5637 cells. Plate colony formation assay and MTT assay were used to evaluate the effects of Nanog over-expression on the proliferation and chemo-sensitivity in 5637 cells. Results The positive rate of Nanog was 50% in bladder cancer tissues, and increased expression of Nanog in bladder cancer was significantly associated with pathological grade (correlation coefficient=0.989,  $P<0.05$ ). Real-time PCR and Western blotting showed that the Nanog expression was obviously higher in the 5637 cells infected with LV-Nanog than those infected with LV-Con lentivirus. The colony formation rate of 5637-C cells and 5637-Nanog cells was  $(4.6\pm0.9)\%$  and  $(9.0\pm1.0)\%$ , respectively, indicating that over-expression Nanog can increase colony formation rate in 5637 cells ( $P<0.01$ ). After cisplatin treatment at 5, 10, 20 and 40  $\mu\text{mol/L}$  for 72 h, the

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survival rate of 5637-C cells was ( $64.5 \pm 4.9\%$ ), ( $53.1 \pm 4.6\%$ ), ( $43.4 \pm 3.9\%$ ) and ( $21.0 \pm 2.7\%$ ) respectively, while the 5637-Nanog cells was ( $80.9 \pm 5.6\%$ ), ( $68.5 \pm 4.2\%$ ), ( $47.9 \pm 5.1\%$ ) and ( $25.2 \pm 4.2\%$ ), respectively. The chemo-sensitivity assay showed exogenous Nanog expression was significantly decreased the chemo-sensitivity of 5637 cells to cisplatin (5 and 10  $\mu\text{mol}/\text{L}$ ) compared with the control cells.

**Conclusion** Nanog protein expression is significantly correlated with the histological grade. Over-expression of Nanog increases the proliferation and decreases the cisplatin sensitivity in 5637 cells.

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