真核表达p27构建胃癌顺铂耐药细胞模型的研究

《现代肿瘤医学》[ISSN:1672-4992/CN:61-1415/R] 期数: 2019年21期 页码: 3766-3769 栏目: 基础研究 出版日期: 2019-09-30

Title: Construction and eukaryotic expression vector of p27 for cisplatin-resistance in SGC-7901

作者: 张寅斌[1]; 陈 琳[2]; 孙诗雨[1]; 陈银溪[1]; 闫婉君[1]; 马宇光[1]; 刘 棣[1]; 梁 亮[1]; 靳耀锋[1]; 张淑群[1]
1.西安交通大学第二附属医院肿瘤科，陕西 西安 710004;2.陕西省人民医院病理科，陕西 西安 710068

Author(s): Zhang Yinbin[1]; Chen Lin[2]; Sun Shiyu[1]; Chen Yinxi[1]; Yan Wanjun[1]; Ma Yuguang[1]; Liu Di[1]; Liang Liang[1]; Jin Yaofeng[1]; Zhang Shuqun[1]
1.Oncology Department，Second Affiliated Hospital of Xi'an Jiaotong University，Shaanxi Xi'an 710004，China；2.Department of Pathology，Shaanxi People's Hospoital，Shaanxi Xi'an 710068，China.

关键词: p27; 克隆; 真核表达; 顺铂; 胃癌细胞; 耐药

Keywords: p27; cloning; eukaryotic expression; cisplatin; SGC-7903; drug resistance

分类号: R735.2

DOI: 10.3969/j.issn.1672-4992.2019.21.005

文献标识码: A

摘要: 目的：构建p27真核表达载体并导入胃癌SGC-7901细胞获得稳定表达p27的稳定细胞株，以研究p27在胃癌SGC-7901细胞顺铂耐药中所发挥的功能。方法：以乳腺文库为模板，PCR扩增出p27编码区，并将其连接到pCDNA 3.0-Flag载体上，转染293T细胞后分别用定量PCR和Western blot检测其表达情况，并通过Western blot检测SGC-7901细胞过表达Flag-p27稳定细胞株是否构建成功。通过CCK-8药物敏感性实验检测p27在胃癌细胞顺铂耐药中所发挥的功能。结果：双酶切和测序结果表明，pCDNA 3.0-Flag-p27构建成功，并在293T中成功表达，胃癌SGC-7901细胞过表达Flag-p27细胞株建立成功，通过耐药曲线表明过表达p27可以引起SGC-7901细胞顺铂耐药。结论：成功构建了带Flag标签的p27真核表达载体，为进一步研究p27在胃癌耐药中的功能奠定了基础。

Abstract: Objective：To construct the eukaryotic expression vector of p27 labeled with Flag tag and detect its function in cisplatin-resistance of SGC-7901.Methods：p27 gene was obtained by PCR from breast library and cloned into pCDNA 3.0-Flag vector.The recombinant plasmid was transfected into 293T cells and identified by either qRT-PCR or Western blot.Meanwhile，SGC-7901 stable cell line transfected with Flag-p27 was detected by Western blot.p27's function in cisplatin resistance of gastric cancer was studied by CCK-8 assay.Results：pCDNA3.0 Flag-p27 eukaryotic expression vector was successfully constructed by double digestion identification，and the inserted fragment was confirmed correct by sequencing.The expression of p27 in human 293T cells and SGC-7901 pCDNA3.0-Flag-p27 stable cell line were successfully identified.Furthermore，drug-resistance curve shows that Flag-p27 overexpression can cause the cisplatin-resistance in SGC-7901.Conclusion：The eukaryotic expression vector of pCDNA3.0 Flag-p27 was successfully constructed and expressed.This study laid a foundation for the further study of drug-resistance in gastric cancer.

参考文献/REFERENCES

[1] Bray F，Ferlay J，Soerjomataram I，et al.Global cancer statistics 2018：GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries [J] .CA：A Cancer Journal for Clinicians，2018，68(6)：394-424.
[2] Sugimoto K，Ito T，Hulbert A，et al.DNA methylation genome-wide analysis in remnant and primary gastric cancers [J] .Gastric Cancer，2019，3(6)：1-12.
[3] Watanabe A，Suzuki H，Kubo N，et al.Stathmin1 regulates p27 expression，proliferation and drug resistance，resulting in poor clinical prognosis in cholangiocarcinoma [J] .Cancer Science，2014，105(6)：690-696.
[4] Escobar-Hoyos LF，Shah R，Roa-Pena L，et al.Keratin-17 promotes p27KIP1 nuclear export and degradation and offers potential prognostic utility [J] .Cancer Research，2015，75(17)：3650-3662.
[5] Yan LH，Wei WY，Cao WL，et al.Overexpression of CDX2 in gastric cancer cells promotes the development of multidrug resistance [J] .American Journal of Cancer Research，2015，5(1)：321.
[6] Simonian M，Mosallayi M，Mirzaei H.Circulating miR-21 as novel biomarker in gastric cancer：Diagnostic

and prognostic biomarker［J］.Journal of Cancer Research and Therapeutics，2018，14(2)：475-476.
［7］Yan J，Dang Y，Liu S，et al.LncRNA HOTAIR promotes cisplatin resistance in gastric cancer by targeting miR-126 to activate the PI3K/AKT/MRP1 genes［J］.Tumor Biology，2016，37(12)：16345-16355.
［8］Deng Z，Wang H，Guo G，et al.Next-generation sequencing analysis of mRNA profile in cisplatin-resistant gastric cancer cell line SGC7901［J］.Med Sci Monit，2019，25(10)：2386-2396.
［9］Moussa RS，Park KC，Kovacevic Z，et al.Ironing out the role of the cyclin-dependent kinase inhibitor，p21 in cancer：Novel iron chelating agents to target p21 expression and activity［J］.Free Radical Biology and Medicine，2019（133）：276-294.
［10］Blagosklonny MV.Are p27 and p21 cytoplasmic oncoproteins［J］?Cell Cycle，2002，1(6)：391-393.
［11］Abbastabar M，Kheyrollah M，Azizian K，et al.Multiple functions of p27 in cell cycle，apoptosis，epigenetic modification and transcriptional regulation for the control of cell growth：A double-edged sword protein［J］.DNA Repair，2018，69(12)：63-72.
［12］Davaadelger B，Duan L，Perez RE，et al.Crosstalk between the IGF-1R/AKT/mTORC1 pathway and the tumor suppressors p53 and p27 determines cisplatin sensitivity and limits the effectiveness of an IGF-1R pathway inhibitor［J］.Oncotarget，2016，7(19)：27511.
［13］Coqueret O.New roles for p21 and p27 cell-cycle inhibitors：A function for each cell compartment［J］?Trends in Cell Biology，2003，13(2)：65-70.
［14］Le TV，Seo Y，Park HJ，et al.Increased expression of p27 is associated with the cisplatin resistance in gastric cancer cell line YCC-3［J］.Archives of Pharmacal Research，2010，33(7)：1127-1132.
［15］Abukhdeir AM，Park BH.P21 and p27：Roles in carcinogenesis and drug resistance［J］.Expert Reviews in Molecular Medicine，2008（10）：e19.

备注/Memo: 陕西省重点研发计划项目（编号：2018SF-226）

更新日期/Last Update: 2019-09-30