[1]耿放,王艾平,陈芳,等.人乳头瘤病毒假病毒体外感染模型的建立及人α防御素5抗病毒作用[J].第三军医大学学报,2012,34(06):473-476.

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人乳头瘤病毒假病毒体外感染模型的建立及人α防御素5抗病毒作用(PDF)

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Title: Establishment of a human papillomavirus pseudovirus *in vitro* infection model and its application to evaluate antiviral activity of human defensin-5

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摘要: 目的 建立用人乳头瘤病毒16亚型假病毒(HPV-16 Psv)感染宫颈癌细胞模型，并研究3种不同构型人α防御素5（HD-5）抗HPV的作用。 方法 将表达HPV-16假病毒衣壳蛋白的重组质粒P^{16L1L2}和含绿色荧光蛋白（GFP）报告基因的质粒P^{fwb}共转染293FT细胞，分离、纯化病毒颗粒后感染宫颈癌细胞株C-33a，同时分别给予20 μg/ml的HD-5/N（天然构型）、HD-5/Acm（半胱氨酸被Acm修饰）或HD-5/Abu（半胱氨酸被Abu替换）处理，培养48 h后荧光显微镜观察和流式细胞分析病毒感染率。 结果 P^{fwb}与P^{16L1L2}成功转染293FT细胞并获得滴度达2.5×10^8TU/ml的HPV-16假病毒液，镜检和流式细胞术检测显示宫颈癌细胞C-33a 能够被假病毒感染并表达GFP。HD-5/N、HD-5/Acm和HD-5/Abu对HPV-16感染C-33a细胞的抑制率分别为(96.48±5.67)%、(69.02±7.88)%和(2.71±1.53)%。与HD-5/N相比， HD-5 /Acm和HD-5/Abu的抗病毒作用显著降低（$P<0.01$）。 结论 建立了基于流式细胞分析的HPV-16假病毒感染模型并将其用于抗病毒药物活性检测。发现HD-5的空间结构和分子中半胱氨酸对其抗HPV感染的活性有重要作用。

Abstract: Objective To establish a human papillomavirus16 pseudovirus (HPV16 Psv) infection model *in vitro* and to determine the anti-HPV effects of human α-defensin 5 (HD-5) with different conformations. Methods The 293FT cells were co-transfected with HPV pseudovirus recombinant plasmid P^{16L1L2} and a GFP reporter plasmid P^{fwb}. After isolation and purification, the pseudovirus particles were used to infect cervical cancer cell line C-33a. During infection, the C-33a cells were treated respectively with 3 different configurations of HD-5, including HD-5/N with natural configuration, HD-5/Acm with Cys residues blocked by acetamidomethyl (Acm) and HD-5/Abu with Cys residues substituted by α-aminobutyric acid (Abu), at a dose of 20 μg/ml. In 48 h later, the cells were observed by fluorescent microscopy and the viral infection rate was determined by flow cytometry assay. Results The 293FT cells were successfully transfected with P^{fwb} and P^{16L1L2}, and 2.5×10^8TU/ml of HPV-16 pseudovirus particles were finally obtained. After culturing, most of C-33a cells were infected by HPV16 pseudovirus, which displayed green fluorescence under fluorescent microscope and could be detected by flow cytometry. The inhibitory rate of HD-5/N, HD-5/Acm and HD-5/Abu on HPV infection was (96.48±5.67)%, (69.02±7.88)% and (2.71±1.53)% respectively. Compared to HD-5/N, antiviral activity of HD-5/Acm and HD-5/Abu was significantly reduced ($P<0.01$). Conclusion A method for HPV16 pseudovirus infection and anti-HPV analysis based on flow cytometry assay is successfully established. The natural configuration of HD-5 contributes to enhance anti-HPV ability significantly, and Cys residues play important role in the antiviral activity of HD-5.

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