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人乳头瘤病毒L1广谱中和表位的鉴定与结构预测定位

Finding, Characterization and Mapping in silico of the Broad-spectrum Epitopes Concealed in Human Papillomavirus L1 Proteins

肖洁琼

指导教师：张军

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摘 要

高危型人乳头瘤病毒(Human papillomavirus，HPV)的持续感染被认为是诱发女性宫颈癌的主要因素。宫颈癌在危害妇女健康的恶性肿瘤疾病中居第二位。世界范围内，宫颈癌每年大约有50万新发病例，死亡人数约20万；中国每年约有13万人罹患宫颈癌。与宫颈癌发生密切相关的高危型HPV有15个型别以上，其中，HPV16与18约占70%。目前，HPV疫苗被认为是防控包括宫颈癌在内的宫颈疾病的最有效手段，而现有的HPV疫苗多因成本与制造工艺困难等因素，仅能覆盖HPV16与18两个型别，无法满足需要。因此，有必要开发一种覆盖型别更广，保护率更高的HPV疫苗。扩大疫苗保护型别、L2疫苗、嵌合颗粒疫苗成为了研究多型别保护的主要方向，而广谱中和单抗及表位的研究是其中最核心的研究内容。

本研究利用多种型别HPVL1 VLP进行了免疫，通过免疫方案及筛选检测方法的摸索，确定了一套成熟的HPV广谱中和单抗的筛选方法：使用不同型别HPVL1 VLP轮流免疫，并使用铝佐剂，免疫剂量为1-10μg/只/次，两次免疫间隔时间为三周。在免疫后26周后进行筛选，筛选时采用ELISA检测与假病毒-细胞中和实验检测相结合的实验方法。经过该方法进行免疫及筛选，得到了三株具有能够中和2种型别以上HPV假病毒的中和单抗，分别为13A10、12B9及4H4。

本研究进一步对三株广谱中和单抗进行了系统的性质鉴定。首先，通过ELISA间接法检测发现：13A10与A9类的HPV58/16/31/33/52 VLP均能反应，其对HPV58五聚体的结合能力较VLP低约500倍；12B9能与HPV16/31/33/58 VLP 反应；4H4能与HPV16/31/33 VLP反应。其次，使用Western-bloting检测发现三株广谱中和单抗都为针对构象表位的单抗；然后，通过多型别假病毒中和实验检测广谱中和单抗的中和滴度，13A10能中和HPV16/58/31/33四种型别假病毒，其中针对HPV16与HPV58的中和滴度高达105，与型特异抗体的中和滴度相当；12B9能中和HPV33/58两型假病毒；4H4能中和HPV16/31/33三型假病毒，中和滴度在103.2至105之间；通过单抗阻断兔多抗血清、病人血清、疫苗注射者血清及型单抗多种阻断实验，其中13A10对单份的HPV58血清的阻断率都在80%以上，并且能够阻断50%以上能与HPV58反应的血清，可以确定13A10为HPV58优势中和单抗；12B9能阻断绝大多数针对HPV33的单抗

，且阻断率较高；但12B9及4H4对血清阻断效果较差，因此确定这两株单抗为非优势中和抗体。最后，本研究还通过生物传感器分析了广谱中和单抗与抗原的亲和力，其中13A10与HPV58L1 VLP亲和力高到10-10mol/L，与型特异性单抗与抗原亲和力相当。

由于13A10是一株能够中和4种HPV假病毒，具有较高的中和滴度与亲和力，而且为HPV58的优势单抗。因此，我们进一步对其表位进行了定位，首先，我们通过GFC-HPLC与AUC的实验方法，确定了13A10与HPV VLP结合的化学计量摩尔比为2.5:1(n:n);然后通过分子模拟的方法，对HPV58五聚体和广谱中和单抗13A10结构进行同源模建和分子对接，预测了13A10与HPV16及HPV58的结合表位：为55-59位aa、442-433位aa 、177及348位aa；并通过HPV16、HPV6L1 loop互换的18种嵌合颗粒验证了分子对接的结果，确定了Ser343、Leu344、Cys345、Ala346、Ile348、Ser349、Thr350中的一个或多个氨基酸为13A10的表位氨基酸。最后，确定Ile348为13A10表位氨基酸。

综上所述，本研究进行了广谱中和单抗制备的免疫方案及筛选方法的摸索，获得三株高滴度的广谱中和单抗，通过ELISA结合、WESTERN-BLOTTING分析、假病毒中和系统检测、阻断实验、BIACORE3000亲和力分析对三株广谱中和单抗进行了系统的性质鉴定；进一步研究了广谱中和单抗的表位。本研究将有益于多价疫苗质控和广谱疫苗的分子设计等。

关键词：人乳头瘤病毒；L1蛋白；广谱中和表位；免疫优势抗体

Abstract

The persistent infection of high-risk HPV usually resulted in cervical cancer, which ranks second in the most common malignant tumors threatening women's health. About 500,000 people are found to suffering from cervical cancer globally, with 200,000 fatal cases each year. And there is about 13 million people suffered from cervical cancer in China yearly. Among the more than 15 kinds of high-risk HPVs involved in cervical cancer, detection rate of HPV16/18 reaches about 70%. HPV vaccine is considered as the most effective means in prophylaxis and control of cervical disease. However, vaccine candidate available at present only cover the HPV16 and 18 due to cost and manufacturing difficulties, which is not good enoμgh for clinical usage. Therefore, the development of vaccines candidate which can prevent more HPVs including HPV16/18 infect could further reduce the incidence of cervical cancer. Multivalent vaccines, L2 vaccine, and chimeric particles vaccine is currently the mainstream of vaccine developments. Thus, the studies of broad-spectrum neutralizing monoclonal antibodies and corresponding epitopes could improve the vaccine development against HPVs.

To screen the broad-spectrum neutralizing monoclonal antibodies of HPV, proper immunizing program was explored in this study, with viral type, state of immunogen, dosage, adjutants, immune intervals and dosage all considered. The final immunizing program established are as following: The crossing immunization using VLPs of different types, dose of 1-10 μg per mouse once and three weeks of immune intervals with Freund's adjuvant. With the combination of ELISA and neutralization detection in hybridoma technique, we get three mAb clones (13A10, 12B9 and 4H4) capable of neutralizing against two or more viral types at last.

We further analyzed the properties of three mAb clones in ELISA, finding that the entire clones bind with VLPs of A9 respectively: clone 13A10 binds with VLPs of HPV58, 16, 31, 33 and 52, clone 12B9 with HPV 116, 31, 33 and 58, clone 4H4

with HPV 16, 31 and 33. Feathers of conformational dependent were identified with Western blot and neutralizing potent against different vial types were analyzed with pseudoviruses neutralization model: clone 13A10 could neutralize with pseudoviruses of HPV16/58/31/33, clone 12B9 with HPV33/58 and 4H4 with HPV16/31/33. The immunodominancy was analyzed in blocking ELISA with rabbit anti sera, the clinical sera of patients infected by HPV, sera of people injected HPV vaccine candidates and type-specific mAbs of HPV. We found that clone 13A10 is immunodominant, which could block half sera of HPV58 with the single blocking rate at 80%. It can be inferred from the poor blocking rate against clinical sera that clone 12B9 and 4H4 were not immunodominant, despite the high blocking rate against HPV 33-specific mAb of clone 12B9. The binding affinity with antigen is analyzed by biological sensor, found that the affinity of clone 13A10 against VLP of HPV 58 is 10-10mol/L, which is equal to type-specific mAbs.

We further analyzed the antigenic sites recognized by clone 13A10 due to the broad-spectrum neutralizing potent, immunodominacy and high binding affinity. Firstly, the proportion of 13A10 binding VLP was detected as 2.5:1(n:n) by GFC-HPLC and AUC. Secondly, structures of HPV58 pentamer and 13A10 were simulated by homology modeling, followed by epitopes prediction in HPV16 and 58. Result shows that the amino acids residue of 55-59,442-433,177 and 348 were involved. Together with mutant protein analysis, the definite amino acids were localized into Ser343,Leu344,Cys345,Ala346,Ile348,Ser349 and Thr350 eventually.At last,we determined that Ile348 is the epitope of 13A10.

Findings from this study provide necessary resources and effective approaches for molecular designing and quality control in universal vaccine development. It also highlights the dominancy of conformational-dependent epitopes in HPV.

Keywords: Human PapillomavirusProtein L1Broad-spectrum neutralizing epitopeImmunodominant antibody

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