CONSTRUCTION OF A RECOMBINANT ADENOVIRUS VECTOR OF HUMAN PAPILLOMAVIRUS TYPE 16 L1_E7C

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ABSTRACT

Objective: Human papillomaviruses are closely associated with human cervical cancer, especially HPV types 16 and 18. At present, HPV can not be produced in large quantity; it also has tumorgenicity and these properties of HPV have seriously hampered the development of HPV vaccine. HPV type 16 L1 proteins can assembled into virus-like particles (VLP), which are morphologically identical to the nature virion. In order to develop the recombinant adenovirus vectors of HPV, we constructed a recombinant adenovirus shuttle plasmid pCA14 L1-E7C. Methods: Human papillomavirus type 16 L1 open reading frame without terminator codon (TAA) (5559–7152) and E7C (682–855) were amplified using PCR. The L1 and E7C fragments were inserted into pGEM-T easy vectors by T–A strategy, named pTAL1 and pTAE7C. pTAL1 was cut with Hind III and BglII, the pTAE7C with BamHI and ClaI. The L1 DNA fragment, E7C and pBluesscript SK were ligated together using T4 DNA ligase. pBSL1-E7C and pBSL1-E7C was digested with Hind III and Xhol. The L1-E7C fragment was inserted into adenovirus shuttle plasmids pCAl4, named pCAl4L1-E7C. DNA sequence results indicated that The L1-E7C DNA fragment can encode the HPV16L1-E7 fusion protein correctly. Results: The L1 and E7C DNA fragments were amplified by PCR and recombinant plasmid pTAL1, pTAE7C, pBSL1-E7C and pCA14L1-E7C were constructed correctly. The pCAl4L1-E7C can be used in the further research work, cotransfected the 293 cell with the parent adenovirus pBHG10. Conclusion: Our results indicated that we have constructed a HPV16L1-E7 fusion DNA fragments and the adenovirus shuttle plasmids pCALl-E7C for the further research.

Keywords: Human papillomavirus, Adenovirus vector, Vaccine

Human papillomaviruses (HPV) that infect the genital tract are associated with human anogenital tract cancer, especially cervical cancer. HPVs are thought to be the primary causative agent in more than >90% of cervical cancers, with type 16 being the most frequently found type in human cervical cancers.[1] Low-risk HPV6/11 infections can cause condylomata through sexual transmission. The advance of research on a candidate prophylactic vaccine against papillomavirus infection has been made. Expression of the papillomavirus major capsid protein, L1 in eukaryotic or prokaryotic cells can self-assemble into virus-like particles (VLP) that are morphologically indistinguishable from native virions and present the conformational epitopes required for the induction of high titer neutralizing antibodies.[2,3] VLP are attractive candidates for prophylactic vaccine against papillomavirus infection. Although VLPs are produced in several eukaryotic and prokayrotic systems successfully, such as baculovirus/insect systems,[4] Vaccinia systems[5] and bacterium,[6, 7] the expressed proteins must be purified from the culture medium or infected cells which is very expensive and tedious. Adenovirus express systems have several advantages in producing HPV vaccines as well as DNA immunization. These systems could offer practical advantages over those in which VLPs are produced directly *in vivo* without the rigorous purification processes and could be used to generate material free of contamination.

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The adenovirus vector has many more advantages. It can normally encapsidate a viral DNA molecule slightly bigger than the normal DNA (105%); it has low pathogenicity in humans. Adenoviruses can infect a broad range of mammalian cells. Our goal was to generate the attenuated recombinant adenovirus carried the human papillomavirus type 16 Ll-E7C fusion gene which can express the Ll-E7C protein at high level. The L1-E7C can self-assemble into papillomavirus-like particles and induce the protective immune reaction against the human papillomavirus infection.

MATERIALS AND METHODS

pHPV16 plasmid (HPV16+pBR322) was a gift from DA Galloway (Seattle, USA), pHPV16L1BN1 was constructed by professor Yu Xiuping. Adenovirus expression system, including the shuttle plasmid pCA14 which carries the HCMV IE promoter and parent adenovirus plasmid pBHG10 were products of Micobix Co. (Canada). Restriction enzymes Bgl II, BamH I, ClaI, Hind III, XhoI, IPTG , X-gal, T4 DNA ligase, Taq DNA polymerase were purchased from GiBco Co. The sequences of primers were as follows: HPV16 L1 primer (upprimer:5'CGCATCGATATGTCTCTTTGGCTGCCT-AG3' downprimer: 5'CCGAGATCTCAGCTTACGTTT-TTTGCG3'), E7C primers (upprimer 5'CGCTGGA-TCCCAAGCAGAACCGGACAG3', downprimer: 5'CG-CGAAGCTTTTATGGTTTCTGAGAACAG-3'). These primers were synthesized by the Cybersyn Corporation (USA), and were modified by adding restriction enzyme recognition sites (BamH I, BglII, Hind III, ClaI). The HPV16 Ll ORF was amplified without termination codon (TAA) (5559–7152) from pHPV16L1BN1. The E7C fragment (685–855) was amplified from HPV16E7 ORF of pHPV16. These fragments were purified by low-temperature melting agarose electrophoresis. The L1 and E7C DNA fragments were inserted into the pGEM-T easy vector respectively. The recombinant plasmids transformed the E.coli DH5α and then were inoculated on the LB-agarose with ampicillin, X-Gal and IPTG. These pTAL1 and pTAE7 plasmids were digested with Hind III / Bgl II and BamH I/Cla I, respectively. The pBluscript SK was linearized with Hind III and ClaI. These DNA fragments Ll, E7 and pBluescriptsk were ligated together using T4 DNA ligase at a ratio of 1:2:4, named pBSL1-E7C. The HPV16 Ll-E7C DNA fragment was cut with Hind III and XhoI, and then inserted into adenovirus shuttle plasmid pCA14, named pCA14L1-E7C.

RESULTS

The HPV16L1 ORF (1593 bp) was amplified from the pHPV16L1BN1 plasmid (5559–7152) without the terminator codon TAA at the 3' terminus of L1 ORF (Figure l). The HPV16 E7C DNA fragment (171 bp) was amplified from the pHPV16 plasmid, which can code the carboxyl domain of E7 protein (57 amino acid) (Figure 2). Those above DNA fragments were inserted into pGEM-T easy vector, pTAL1 and pTAE6, correctly. In order to make the L1-E7C fusion DNA fragment, the L1 and E7C DNA fragments released from pTAL1 and pTAE7C, together with the linearized pBluescript SK were ligated by T4 DNA ligase at a ratio of 1:2:4; DNA sequencing results suggested that the three DNA fragments were ligated at correct orientation as designed. The fusion fragment can encode fusion protein L1-E7 with 590 amino acids with two additional amino acids which were encoded at the added restriction enzyme recognition sites (BamHI and Bgl II ATAGCC). The L1-E7C DNA fragment was released with Hind III and Xhol and then inserted into adenovirus shuttle plasmid pCA14, named pCAl4Ll-E7c. Restriction enzyme digestion results suggested that the pCA14Ll-E7 gene can express L1-E7 fusion protein correctly (Figure 3). So we can do further cotransfection 293 cells with pBHG10 using the shuttle plasmid pCA14L1-E7C.

Fig. 1. The HPV16L1 open reading frame without terminator codon taa (5559–7152) was amplified from the pHPV16L1BN1 using polymerase chain reaction, marker Lamda DNA/Hind III; Lane l: HPVI6L1 ORF without terminator codon from pHPV16L1BN1; Lane 2, 3, 4: HPV16 L1 ORF, amplified from the recombinant adenovirus of HPVI6 infected 293 cells; Lane 5: The E7C fragment amplified from the recombinant adenovirus of HPV16 L1-E7C; Lane 6: Negative control.

DISCUSSION

Papillomaviruses are non-enveloped, double-stranded DNA viruses that infect a range of mammalian species. At present, about 80 types of papillomaviruses have been identified. The HPV type 16 and 18 were associated closely with the development of human cervical cancer. HPVs can induce hyperproliferative lesions of the cutaneous and mucosal epithelia. Two groups of genital HPVs has been classified: low-risk HPV6/ll induce genital warts, high-risk HPV 16/18 closely associated

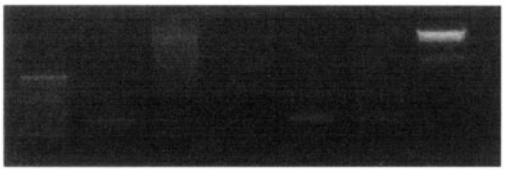

Fig. 2. The HPV16 E7C DNA fragment (685–855), amplified from the pHPV16 plasmid using polymerase chain reaction. M: DNA marker Lamada DNA /Hind III, Lane 1, 2. The E7C DNA fragment amplified from the pHPV16, Lane 4, 5. The E7C DNA fragments amplified from the recombinant adenovirus of HPV16 infected 293 cells. Lane 3 was negative control pBR322.

with cervical cancer. It has been found that HPV major capsid protein L1 (alone or with minor capsid protein L2) can self-assemble into papillomavirus-like particle (VLP) when expressed in eukaryotic and prokaryotic cells. It is known that in the carboxyl domain about 30 amino acids are not necessary to its assembly into the virus-like particle, and heterologous short peptide can be inserted into the region.[8] Immunization with VLPs leads to high titer production of HPV neutralizing antibodies and MHC-I restricted CTL effect.[9–13] So the VLP become a promising prophylactic vaccine candidate against human papillomavirus infection and HPV-associated neoplasm. But they are unlikely to have therapeutic effects because the virion capsid proteins are not formed in the proliferating cells of the infected epithelia or cervical cancer cells. HPV early protein E7 has transforming ability and plays an important role in the tumorigenicity of HPV.[14] Some research results indicated that the E7 protein major epitopes were clustered at the carboxyl region. It has been shown that E7N region has the binding ability to binding the tumor suppressor protein pRB, which abrogate the tumor suppressor ability of pRB protein by dissolving the complex of pRB and E2F transcription factor. The adenovirus expressed L1-E7C fusion protein can form chimeric virus-like particles in human cells which have both prophylactic and the therapeutic effects against HPV infection and HPV associated neoplasm. The development of human papillomavirus faces a serious challenge, based on the following problems: Firstly, human papillomavirus can not be produced in large quantity *in vitro*, so it is difficult to obtain enough virions to do further research; secondly, it has been difficult to develop HPV vaccines because high-risk HPVs have oncogenic DNA genomes; thirdly, papillomavirus infections are species restricted. There are no animal models for HPV infection for experimental infection. Before beginning clinical trials it was necessary to develop serological assay to measure the immune response to HPV VLP vaccination. The gold standard is an *in vitro* antibody neutralizing assay; it is very difficult to develop these assays for high-risk HPVs because they can not be efficiently propagated and don't induce readily detected changes in infected culture cells.[15]

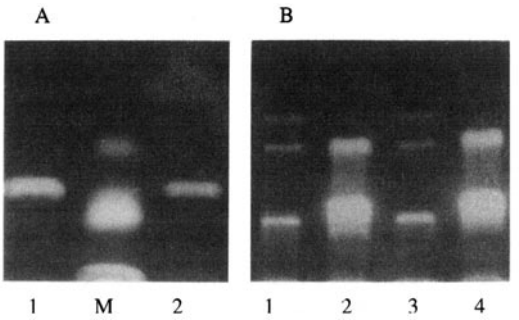

Fig. 3. The restriction enzyme digestion result of the recombinant plasmid pCA14L1-E7C. A: Lane 1 and Lane 3. pCA14L1-E7C cut with BamH I and Hind III produced three DNA fragments: 6.95 kb, 0.94 kb, 0.78 kb, because there were 2 BamH I sites in multicloning site and the L1-E7C DNA fragment; M: Lamada DNA/Hind III DNA marker. B: Comparison of DNA fragment between the cut pCA14L1-E7 plasmid with the enzymes described above and the non-cut plasmids pCA14L1-E7C. Lane 1, 3. Non-cut pCA14L1-E7 plasmids, Lane 2, 4. pCA14L1-E7 plasmids cut with the enzymes described above.

The adenovirus can normally encapsidate a viral DNA molecule slightly bigger than the normal DNA (105%). The El and E3 early regions of the adenovirus have been deleted to provide more room for cloning. The expression system has many more advantages: 1. Replicates can efficiently reach high titers about 10^8 to 10^{11} PFU/ml. 2. Broad host range and low pathogenicity in humans. The adenovirus expression system has been used extensively to express human as well as non-human proteins. 3. Adenoviruses can infect a broad range of mammalian cells and therefore allow the expression of recombinant proteins in most mammalian cell lines and tissues. 4. Homologous system for human genes. The adenovirus expression system uses a human virus as a vector and human cells as host cells. It therefore provides an ideal environment for proper folding and precise posttranslational modifications of human proteins. Most human proteins are expressed at a high level and are fully functional. Adenovirus can infect all types of cells except some lymphoid cells. Adenovirus is the best system to study the expression of genes in primary non-replicating cells. This allows for a direct comparison of results obtained with transformed cell lines and primary cells. Retroviruses integrate randomly into the host chromo-

some and can inactivate genes or activate oncogenes. For gene therapy, adenoviruses remain epichromosomal in all known cells except eggs and therefore do not interfere with other host genes. The yield of recombinant proteins can be up to 10–20% of total cellular proteins (TCP). Based on these criteria, it might be the best vector system for gene therapy applications.[13] Combined the great advantages of adenovirus vector, the papillomavirus virus-like particles vaccine will give much more protective immune reaction against the human papillomavirus infection.

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