Human papillomavirus type 6 and 11 E4 gene products in condyloma acuminata

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Human papillomavirus (HPV) is a diverse group of DNA tumour viruses that cause hyperproliferation of the cutaneous and mucosal epithelia. The genome organization of HPVs is very similar, but HPV infection is highly tissue-specific. HPV-1, -2 and -4 induce skin warts and HPV-6 and -11 have been known to induce anogenital tumours such as condyloma acuminata. HPV gene products and their functions have been extensively studied in vivo and in vitro. Doorbar et al. (1986, 1988) and Breitburd et al. (1987) identified HPV-1 E4 gene products which occur mainly as 16K and 17K proteins in warts and accumulate to become up to 30% of the total protein of the warts. The E4 gene products of HPV-2 and -4 in natural tumours have also been reported to occur as doublets of 16.5K/18K and 20K/21K, respectively. These proteins (Doorbar et al., 1989) as well as a predicted HPV-16 E4 protein (Jochmus-Kudielka et al., 1989) have been reported to have highly specific antigenicity.

At present, the precise function(s) of the E4 protein in viral replication and in tumour formation is (are) not known. To clarify the function of HPV-6 and -11 E4 proteins, we characterized initially the E4 gene products in condyloma acuminata. Here, we report that the E4 proteins occur as 11K or as 10K/11K doublets in genital condylomas and that the E4 proteins of HPV-6 and -11 have a cross-reactive antigenicity. Furthermore, we report a possible proteolytic conversion of the 11K protein to the 10K species.

Nasseri et al. (1987) cloned a cDNA corresponding to the 1-2 kb HPV-11 E4 mRNA which was derived from an in-frame splice of the E1 and E4 exons. On the basis of the high similarity between the HPV-11 and HPV-6b DNA sequences, they predicted HPV-6 El/E4 mRNA, which has a typical splice donor site at nucleotide (nt) 847 and a splice acceptor site at nt 3325. To construct an expression vector for the HPV-6 E4 protein in Escherichia coli, we synthesized an oligonucleotide, ATGGCG-GACGATTCAGCACTACACAAGAAG, corresponding to the HPV-6b sequence (de Villiers et al., 1981) and used it as a primer for site-directed mutagenesis. After cloning the E4 cDNA fragment containing nt 832 to 847/3325 to 3581 in pKK233-3 (Pharmacia), a lacZ fragment from pMC1871 (Pharmacia) was ligated into the plasmid and pKKE4lacZ was obtained (Fig. 1a). The plasmid was transfected into E. coli JM109 cells and the E4-β-galactosidase fusion protein (E4-β-Gal) was induced by isopropylthio-β-D-galactoside. Then the fusion protein was purified by affinity chromatography with ProtoSorb LacZ (Promega Biotec) (Fig. 1b). Correct expression of the fusion protein was verified by determining the sequence of the 18 N-terminal amino...
Fig. 1. Expression of the E4–β-Gal fusion protein in E. coli and preparation of the anti-E4–β-Gal antibody. (a) Construction of the E4–β-Gal expression vector (pKKE4lacZ). ptac, tac promoter; lacZ, E. coli β-galactosidase gene. (b) SDS-PAGE (7.5% gel) analysis of E. coli JM109 cell lysates. Lane 1, E. coli JM109; lane 2, E. coli JM109 transformed with pKKE4lacZ; lanes 3 and 4, affinity-purified E4–β-Gal. (c) Western blotting of E4–β-Gal protein. Lane 1, preimmune rabbit serum; lane 2, hyperimmune serum; lane 3, anti-β-galactosidase antibody (Promega Biotec). These primary antibodies were used at a dilution of 1:400 (lanes 1 and 2) or 1:1000 (lane 3). Arrowheads indicate the E4–β-Gal protein.

Fig. 2. (a) Western blot analysis of HPV-6 and -11 E4 gene products in tissues. Lane 1, normal epithelium; lanes 2 to 5, condyloma specimens C-1, C-8, C-13 and C-14, respectively. Anti-E4–β-Gal serum was used at a 1:100 dilution. (b) Western blotting of the C-1 tissue lysate. Anti-E4–β-Gal serum was preincubated with the E. coli lysate (Promega Biotec) (lane 1) or with the E. coli JM109 cell lysate containing the E4–β-Gal (lane 2). (c) Southern blot analysis of PstI-digested total DNA (3 gg) from condylomas. Lanes 1 to 3: DNAs from C-1, C-8 and C-13, respectively. A mixture of 32P-labelled HPV-6 and HPV-11 DNA was used as the probe.

Using the anti-E4–β-Gal serum, we analysed 18 condyloma specimens by Western blotting as described previously (Tomita et al., 1987), and six specimens were specifically recognized by the anti-E4–β-Gal serum. Four out of the six specimens contained two protein species which migrated to 10K and 11K in SDS-PAGE. In two specimens, C-8 and C-23, only the 11K protein...
was detected, but in specimens C-1, C-13, C-14 and C-19 10K/11K doublets were detected as shown in Fig. 2(a) and Fig. 3(a). Although the ratio of 11K to 10K differed, the 11K protein was major in each case. The binding of the anti-E4-β-Gal serum with these proteins was probably specific because these protein species were not found in the normal epithelium (Fig. 2a) and the preimmune serum did not react with these proteins (data not shown). Furthermore, binding of the anti-E4-β-Gal antibody to the 10K/11K doublets was inhibited by preincubation of the serum with E. coli lysate containing E4-β-Gal, but not with the E. coli lysate lacking the fusion protein (Fig. 2b). From these results and from the Mr, which coincides with that calculated from the length of the putative cDNA, we concluded that these proteins were HPV-6 E4 proteins in natural condyloma tissues. In addition to the specific reaction, the anti-E4-β-Gal serum reacted with the 17K protein in some condyloma tissues (Fig. 2a). However, this reaction seemed to be non-specific because the anti-E4-β-Gal serum reacted with the 17K protein in the normal epithelial tissue (Fig. 2a) and in some condylomas which lack the 10K/11K doublet (data not shown). Furthermore, the same reaction was observed when the preimmune rabbit serum was used at a low dilution (data not shown).

The similarity between the predicted E4 protein of HPV-6 and that of HPV-11 has been estimated to be about 80% (Nasseri et al., 1987), and both proteins have been expected to have a closely related antigenicity. To demonstrate the antigenic cross-reactivity, we analysed HPV types in 11 condyloma tissues and found that nine condylomas harboured HPV-11 and two harboured HPV-6. As shown in Fig. 2(c), the specimen C-13 contained HPV-6 DNA, but specimens C-1 and C-8 contained HPV-11 DNA. These results show that the E4 proteins of HPV-6 and -11 are specifically recognized by the anti-HPV-6 E4-β-Gal serum.

After repeated freezing and thawing of tissue, we noted a minor 10K protein in a condyloma in which originally only the 11K protein had been detected. To examine the possible conversion of 11K protein to 10K in vitro, one condyloma specimen, C-23, was minced and incubated at 37°C. As shown in Fig. 3, the original C-23 tissue contained only the 11K protein, but after repeated freezing and thawing and incubation at 37°C, the 10K protein clearly appeared, suggesting the in vitro conversion of the 11K protein into the 10K protein. These results also suggest that the same conversion could occur in condylomas in vivo.

Based on the common physical properties of the E4 proteins of HPV-1, -2 and -4, Doorbar et al. (1989) proposed that the conserved function of these E4 proteins is probably related to the productive stage of viral infection. Namely, in natural tumours, E4 proteins of HPV-1,-2 and -4 occur as doublets (HPV-1 16K/17K; HPV-2 16.5K/18K; HPV-4 20K/21K). The E4 gene products of the HPV-11 Hershey isolate have also been reported to occur as a 10K/11K doublet in human tissue implanted under renal capsules of athymic nude mice (Brown et al., 1988).

In condylomas, however, the 10K species was minor in amount compared with the 11K species. Furthermore, in condyloma specimens C-8 and C-23, only the 11K species was detected. In addition, the 10K protein species was found in the same tissue (C-23) after repeated freezing and thawing and incubation at 37°C. Therefore, it is likely that the 10K protein is a secondary product derived from the 11K protein in vivo and in vitro by a post-translational modification such as proteolytic cleavage, as suggested by Doorbar et al. (1989) and Palermo-Dilts et al. (1990).

The E4 proteins were detected in only six out of 18 condylomas. This may be due to differences in the growth stage of the tumour in each specimen, which may correspond to the accumulation and degradation of the E4 protein.

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References


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