

Study of infectious virus production from HPV18/16 capsid chimeras

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ARTICLE INFO

Article history:

Received 1 February 2010
Returned to author for revision 13 May 2010
Accepted 17 May 2010
Available online 3 July 2010

Keywords:

Papillomavirus
Chimera
Organotypic culture
Raft culture
Capsid genes
Virion morphogenesis

ABSTRACT

Using the HPV18 genome as the backbone, we exchanged the HPV18 L2 or L1 genes with those of HPV16. The intertypical exchange of HPV18 L1 with the HPV16 L1 produced genomes that efficiently replicated and produced infectious virus. Genomes containing an intertypical exchange of HPV18 L2 for the HPV16 L2 failed to produce infectious virus in multiple independently derived cell lines. Using chimeric constructs of individual capsid proteins, we identified a type-specific domain at the N-terminus of the HPV18L1 capsid protein, which interferes with its ability to cooperate with the HPV16 L2 protein to form infectious viral particles. Deletion of this domain allows for the cooperation of the HPV18 L1 protein and HPV16 L2 protein and production of infectious progeny. In addition, cooperation of this N-terminal HPV18 L1 deletion mutant protein with the wild-type HPV18 L2 protein efficiently replicates infectious virus but changes occur in the viral structure.

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Introduction

The life cycle of human papillomaviruses (HPV) has evolved to be intimately connected to the differentiation program of host epithelial tissues (Meyers et al., 1992; Meyers et al., 1997; Taichman and LaPorta, 1986). The development of the organotypic (raft) epithelial culture system has allowed for the development on an in vitro culture system capable of reproducing the complete HPV life cycle, including the propagation of infectious viral particles (Alam et al., 2008; McLaughlin-Drubin et al., 2004; McLaughlin-Drubin and Meyers, 2004; McLaughlin-Drubin et al., 2003; Meyers et al., 1992; Meyers et al., 1997; Sen et al., 2004). The raft culture system has been used to study in detail the steps in the HPV life cycle (Alam et al., 2008; Bedell et al., 1991; Bodily et al., 2006; Bodily and Meyers, 2005; Frattini et al., 1996; Grassmann et al., 1996; Mayer and Meyers, 1998; Ozbun and Meyers, 1996; Ozbun and Meyers, 1997; Ozbun and Meyers, 1998a,b; Ozbun and Meyers, 1999b; Sen et al., 2004). In addition, two recent publications have shown that the replication and maturation of native virus in differentiating host tissue differs in significant aspects from particles made using pseudovirus/quasivirus technologies (Conway et al., 2009a,b).

Chimeric viruses are commonly used to compare genes from one virus with the homologous genes from a related virus to determine the similarities and differences of those genes. A chimeric virus system can be used to assign a particular viral phenotype to a specific gene or sequence. Another use of a chimeric virus system is to discover the commonalities of related viral genes. In this article, we used chimeric HPVs to test the hypothesis that, although the HPV18 and HPV16 capsid genes have a high amount of sequence homology, there are type-specific domains affecting the interaction of the two capsid proteins during virion morphogenesis.

Previously, we designed experiments to study whether the non-structural genes from one HPV type could function with the structural genes of another HPV type. In that study, we replaced the L1 and L2 capsid protein open reading frames (ORFs) from HPV type 18 (HPV18) with the L1 and L2 capsid protein ORFs from HPV16 (Meyers et al., 2002). The resulting HPV18/16 chimeric virus was able to carry out the complete viral life cycle culminating in the production of infectious virus after introduction into keratinocytes that were allowed to terminally differentiate and stratify in raft culture. Antiserum raised against HPV16 virus-like particles (VLPs) and not HPV18 VLPs specifically neutralized the HPV18/16 chimeric virus (Meyers et al., 2002). This study established the use of a viable chimeric virus replication system to study HPV genetics and confirmed the ability of the nonstructural genes of HPV18 to function with the structural genes of HPV16.

To extend these studies, we replaced either the L1 capsid ORF of HPV18 with that of HPV16 L1 or the L2 capsid ORF of HPV18 with that of HPV16 L2. Chimeric HPV18 containing the HPV16 L1 ORF were able to behave similar to wild-type HPV18 in that they completed the viral life cycle in terminally differentiating raft tissue with the production of infectious chimeric viral particles. However, while chimeric HPV18

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containing the HPV16 L2 ORF immortalized primary keratinocytes was maintained in an episomal state, induced a transformed phenotype in raft culture, amplified viral DNA (vDNA), and HPV18 L1 and the HPV16 L2 capsid gene expression appeared normal in raft cultures, we were unable to detect the production of infectious virus from this chimera. In an effort to begin to narrow down the region of the HPV18 L1 or HPV16 L2 capsid gene responsible for the interference of production of infectious particles, we analyzed chimeric exchanges of only half of the L1 or L2 capsid gene ORF of HPV18 with that of HPV16. These studies identified the N-terminus of the L1 capsid protein of HPV18 to be inhibitory to the production of infectious chimeric virus containing the HPV16 L2 capsid gene.

Results

L2 and L1 chimeric viruses

Previously, to investigate whether the nonstructural genes of one HPV type could cooperate with the structural genes of a second HPV type during the complete viral life cycle, we constructed a recombinant plasmid consisting of the URR and the nonstructural early gene ORFs of HPV18 and the structural late gene ORFs of HPV16 (Meyers et al., 2002). This chimeric virus behaved similar to wild-type HPV18 or HPV16 maintaining 50–100 episomal genomes copies in infected cells; on differentiation in the organotypic raft culture system, late viral functions, including capsid gene expression and infectious virus propagation, were observed (Meyers et al., 2002). These data demonstrated that the nonstructural genes of HPV18 function with the structural genes of HPV16, allowing the complete HPV life cycle to occur. In the present study, we hypothesized that the structural genes of one viral type contain domains that may affect intertype interactions during the viral life cycle and virion morphogenesis.

To test this hypothesis, it was necessary to create chimeric mutant viruses exchanging either the L2 or the L1 ORF of HPV18 for the L2 or L1 ORF of HPV16. An initial problem with creating these chimeric mutants is that the L2 and L1 ORFs of HPV18 and HPV16 overlap; therefore, direct exchanges of one of the ORF would affect the other ORF (Fig. S1). To overcome this situation, we first created mutant viruses using PCR technology to individually amplify each ORF, introducing appropriate restriction enzyme sites and cloning the amplicons into the pHPV18L1/L2Δ plasmid that lacks the L2 and L1 ORFs (Meyers et al., 2002) (Fig. S1). This created two mutant viral genomes; one wild-type for HPV18 except that the L2 and L1 ORFs do not overlap but are now separated by a unique *HindIII* site, the second is similar to our previous reported HPV18/16 chimera (Meyers et al., 2002), except that the HPV16 L2 and L1 ORFs do not overlap but are now separated by a unique *HindIII* site (Fig. S1). We named these mutants HPV18-L2(18)L1(18) and HPV18-L2(16)L1(16). Our main goal was to determine if capsid proteins from two different HPVs could cooperate to produce infectious virus, and if not, then what domain(s) of the protein is responsible. However, in creating these mutant genomes, there was the possibility that the tandem

duplication of the putative L1 splice acceptor would interfere with expression of the capsid protein and therefore the ability of these mutants to produce infectious virus. Therefore, we first analyzed the mutants for their ability to correctly express the capsid proteins and produce infectious virus.

Continuously infected cell lines were made using our standard electroporation protocol previously reported (Conway et al., 2009a,b; McLaughlin-Drubin et al., 2004; McLaughlin-Drubin and Meyers, 2004; McLaughlin-Drubin and Meyers, 2005; McLaughlin-Drubin et al., 2003; Meyers et al., 2002; Meyers et al., 1997). Upon differentiation in raft culture, these cell lines were tested for late viral functions of viral genome amplification by Southern blotting, capsid gene expression by Western blotting, and propagation of infectious progeny by a limited dilution RT-PCR titrating assay. Wild type and mutant viruses had a similar phenotype including viral titers when late viral functions were analyzed (Fig. 1). This suggested that the physical separation of the L2 and L1 ORFs had no significant effect on the viral life cycles. We concluded that construction of mutant viruses having their capsid gene ORFs separated did not introduce any adverse effects in the capsid protein expression and the production of infectious virus. All mutant genomes for this section and throughout this report were analyzed by restriction digestion and sequenced to ensure they were correct. Here and throughout the article, three or more independently derived virus-infected cell lines were tested, and the results were always found to be similar.

We then proceeded to create two chimeric mutants by exchanging the HPV18 L2 or L1 ORF for its counterpart from HPV16 (Fig. S1). We named these mutants HPV18-L2(18)L1(16) and HPV18-L2(16)L1(18). Cell lines continuously infected with each mutant were made as described (Conway et al., 2009a,b; McLaughlin-Drubin et al., 2005; McLaughlin-Drubin et al., 2004; McLaughlin-Drubin and Meyers, 2004; McLaughlin-Drubin et al., 2003; Meyers et al., 2002; Meyers et al., 1997). Upon differentiation in raft culture, these cell lines were tested for late viral functions of vDNA amplification by Southern blotting, capsid gene expression by Western blotting, and propagation of infectious progeny by Limited Dilution RT-PCR titrating assay. Both mutant viruses were similar to wild-type in their ability to amplify their genomes and express their capsid genes, however, when infectivity was measured by Limited Dilution RT-PCR titrating, HPV18-L2(18)L1(16) was capable of infectious virus synthesis similar to wild-type, but the chimera HPV18-L2(16)L1(18) was not (Fig. 1). Three individual continuously infected cell lines using three separate batches of primary foreskin keratinocytes were originally made for each of the mutants. To rule out the possibility that the particular genetic background of the three batches of primary foreskin keratinocytes were responsible for the lack of infectious virus production, we created 17 more HPV18-L2(16)L1(18) continuously infected cell lines for a total of 20 cell lines. Infectious virus was undetectable in 19 of the 20 cell lines after growth in raft culture (Table 3). Following growth in raft culture, we were able to detect infectious virus with only 1 of the 20 cell lines, but detection was at

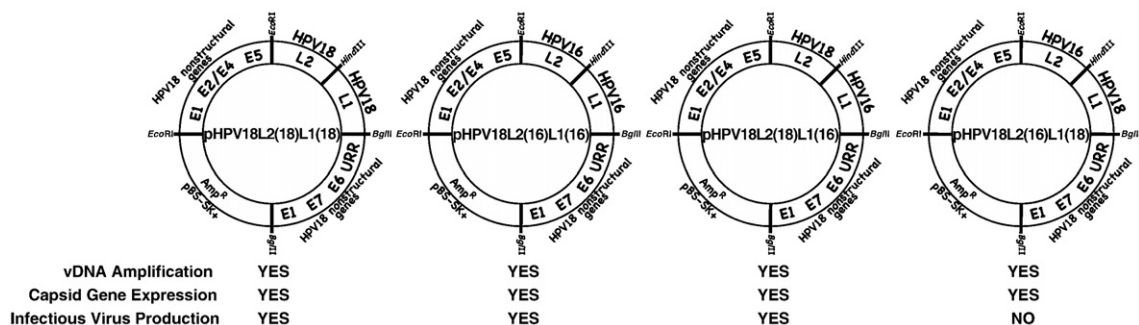


Fig. 1. Late functions of viral mutants with nonoverlapping L2 and L1 ORFs. Late viral life cycle functions were analyzed for the four mutant HPV18 viruses containing nonoverlapping L2 and L1 ORFs; HPV18-L2(18)L1(18), HPV18-L2(16)L1(16), HPV18-L2(18)L1(16), and HPV18-L2(16)L1(18).

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