

A Novel Human Papillomavirus Sequence from an International Cervical Cancer Study

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Novel human papillomaviruses (HPVs) were sought as part of a recent international study of >1000 invasive cervical carcinomas. A single novel HPV designated IS39 was identified in this study. IS39 is most closely related to another novel virus, W13B (MM4), variants of W13B (MM4), and HPV-51.

Genital human papillomaviruses (HPVs) are considered common sexually transmitted disease agents and represent the central etiologic agent for cervical neoplasia worldwide [1–3]. Specific HPV types (e.g., HPV-16, -18, -31, -33, and -45) have been shown to be associated with cervical intraepithelial neoplasia and carcinoma, while other types (e.g., HPV-6 and -11) are rarely associated with severe grades of cervical dysplasia [3, 4]. The involvement of less prevalent, unidentified HPVs in cervical cancers was investigated as part of this study.

Recent combined efforts to detect novel HPVs in cervical scrape, swab, or lavage specimens from women with normal or dysplastic cytologic diagnoses have resulted in the identification of 9 novel HPVs as described [5–8]. We describe here a single novel HPV identified in an invasive cervical carcinoma biopsy from the International Biological Study of Cervical Cancer (IBSCC) [3].

Methods

Amplification using polymerase chain reaction (PCR) was done with L1 consensus primers (MY11 and MY09 plus analogous type 51-specific primer) as described [9, 10].

Specimens that hybridized with the generic HPV probe but not with any of the type-specific oligonucleotide probes were subjected to restriction fragment length polymorphism (RFLP) analyses as described [8, 11]. Specimens that did not yield discernible patterns were taken directly to cloning and sequencing.

The ~450-bp PCR fragments of interest were cloned directly into pT7Blue (Novagen, Madison, WI) and transformed into NovaBlue cells (Novagen). Plasmid minipreps of the transformed cells were subjected to alkali denaturation and double-stranded dideoxy sequencing (Sequenase 2.0; United States Biochemicals, Cleveland) with vector-specific primers flanking the MY09/MY11 fragment insert. The nucleotide sequence was determined from multiple clones. Nucleotide and amino acid sequence similarities of the MY09/MY11 L1 fragment to reference strain HPVs were determined by the FASTA program (Genetics Computer Group, Madison, WI) after removal of the primer-derived sequences. Sequences have been deposited in GenBank with accession numbers U12481 (IS39), U12483 (IS766), U12484 (IS887), and U12482 (IS1016).

Results

Identification of a novel HPV. Previously characterized HPV types were detected in most (>99%) of the 837 IBSCC invasive cervical carcinoma specimens that were positive for HPV DNA [3]. Twelve specimens yielded an HPV amplification product that did not hybridize with type-specific oligonucleotide probes. After cloning and DNA sequence analysis of the ~450-bp PCR products, several HPVs were detected that had not been probed for in the IBSCC, including HPV-64, -67, and -69 and a novel HPV designated CP8061 [5]. Two HPV-45 variants were identified. DNA sequence information from the MY09/MY11 L1 fragment of these HPV-45 variants demonstrated nucleotide differences within the probe binding sequences. Two specimens, designated IS39 (from Argentina) and IS215 (from Cuba), yielded identical

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