A Closer Look at Papillomavirus Variants

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Associations between human papillomavirus (HPV) and cervical intraepithelial neoplasia (CIN) have been consistently reported with relative risks in the range of 20-70 [1-5]. In longitudinal studies, persistent repeated detection of cancer-associated HPVs [6] is a strong risk factor for the development of cervical intraepithelial neoplasia (CIN) [7, 8]; high viral load is also presumably a contributing factor [9]. HPV 16 is associated with the majority of cervical cancers worldwide [10], and thus provides the greatest opportunity to further consider biological modifiers of HPV- associated disease risk. As factors affecting the risk for HPV-associated cervical disease are identified, the complexity of the research questions becomes apparent. Host immunologic factors appear to be important as modifiers of risk; however, the role of sequence variation within HPVs as it may relate to persistence or risk for CIN and invasive cancer has not been thoroughly considered.

Currently, HPV genotypes have been used as the exposure measure for HPV-associated risk for invasive cervical carcinoma. This level of exposure determination has not yet provided us with useful prognostic markers that are markedly better than cytologic screening for determining risk of cervical disease progression. Unlike many other viral systems, the use of a serologic typing scheme based on antibody recognition of viral capsids and disease relevance has not been possible for HPVs as viral particles have been unavailable for these types of characterizations. Thus, papillomavirus (PV) type designations were originally based on a less than 50% nucleic acid relatedness across an approximate 8 kb genome in liquid hybridization experiments [11-13]. PV types were therefore considered and assigned based on criteria that emphasized extreme differences rather than relationships. Currently a PV genotype is defined as a viral DNA having less than 90% nucleotide similarity to any other PV genotype in the L1 open reading frame (ORF); a PV subtype is defined as a viral DNA having between 90 and 98% similarity; and a PV variant is defined as a viral DNA having more than 98% nucleotide similarity [14]. Using the above definitions, the large number of disease-relevant HPV genotypes identified during the past decade has posed a challenge to the development of simplified HPV detection systems [15]. Consequently intratypic variation and the relationships between HPVs of the same genotype have not been widely considered.

While most sampling and sequencing efforts have been biased towards a search for more divergent genomes [15, 16], recent investigations have failed to identify intermediate levels of divergence. HPV subtypes have been rarely found: these include subtypes of HPV 5 and 8, the HPV 34-HPV 64 pair, the HPV 44-HPV 55 pair, and HPV 68 and the genome identified in the ME180 cell line [15, 17, 18]. Thus, the overall distribution of currently circulating HPV DNAs is one of clusters of closely related genotypes (variants). These variant clusters are usually significantly separated from each other.

For the most part, the focus has been concentrated on HPV variants although variants of bovine papillomavirus have been reported [19]. Studies of sequence variation in epidermodysplasia verruciformis (EV)- related HPVs have emphasized HPV 5 and HPV 8 [17, 18]. For genital HPVs, studies of variants have been directed at establishing intra- and intertypic phylogenetic relationships with sequence-specific information primarily limited to studies of HPV 16 and HPV 18 [20-35]. A few of these studies have examined sequence-specific changes and disease associations, but these investigations were limited to the E2, E6, E7 and/or L1 ORFs [20, 24, 31–33].

Variants of HPV 16 appear to be stable, since identical variants have been found in unrelated individuals residing in different countries who have no known contact with each other . For HPV 16, five distinct phylogenetic branches have been reported [22, 26, 27]. These branches have been designated E (European), As (Asian), AA (Asian-American), Af1 (African-1), and Af2 (African- 2). Figure 1 summarizes the distribution of these five HPV 16 variant lineages reported in the worldwide study of Ho and coworkers. Recent studies conducted in U.S. populations and in 22 countries support

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the notion that representative variants from all of these five major HPV 16 lineages are detected in populations worldwide, although specific prevalences differ by geography [35, 36]. Differences in the geographic distribution of HPV variants may be explained by founder effects and/or may reflect variant selection in ethnic groups.

Studies of variation in different regions of the HPV 16 genome (e.g., LCR, E6, L1, and L2) indicate that recombination between variants is rare or perhaps nonexistent [20, 35, Part III of this compendium]. These data imply that nucleotide changes in one region of the genome can be used as markers of changes found in other regions within the same lineage. Nucelotide and amino acid variation occurs more frequently in some regions of PV genomes than others [26, 35]. This observation likely reflects functional constraints on the more conserved regions. Current data indicate that the HPV 16 L2 ORF is more varied than the other coding regions and is approximately as varied as the LCR while L1 is the most conserved region [35]. With regard to the LCR, the overall rate of mutation in the reported sequenced region falls between the synonymous and nonsynonymous rates of substitution for the coding regions. This suggests that the LCR segment, while under less selectional pressure than the coding regions is far from unconstrained. Recent data from twelve distinct HPV types (including HPVs 18, 33, 35, 39, 45, 51, 52, 58, 59, 68, MM9, and MM4) indicate that regions of greater intratypic variability correspond to regions of greater intertypic variability [37]. This observation suggests that at least some of the factors that determine establishment of variants are the same as those which determine the establishment of genotypes.

Direct inspection of the HPV 16 nucleotide sequence alignments in Parts I and II demonstrates sites at which there are three or four different base substitutions. These sites can be observed in different lineages and may represent mutational or selection hotspots. A possible explanation for concentrations of nucleotide substitutions could be selective immune pressures. In this regard, the amino terminus of the HPV 16 E6 region represents an interesting region for further consideration. Immunological studies have identified both humoral and cellular immune responses to the HPV 16 E6 protein [33, 38, 39, 40]. The relevance of the E6 N-terminal region is supported by demonstration of an endogenously processed HLA A*0201-restricted peptide (KLPQLCTEL; E6 aa 11 to 19) [38] as well as an overlapping HLA B-7 restricted peptide (RPRKLPQL; E6 aa 8 to 15) in this region [33]. It is plausible that host factors, such as presenting HLA molecules, and viral factors, such as HPV sequence variability, may be important to further defining HPV-associated disease risk.

At least one HPV variant with significantly altered functional properties has been isolated [41, 42]. The L1 protein from the 114K variant of HPV 16 efficiently assembles into virus-like particles while L1 expressed from the reference HPV 16 genome does not. Kirnbauer and coworkers found that a single amino acid change at residue 202 (Asp to His) most likely resulted in this assembly difference. It will be interesting to determine if HPV 16 E6/E7 variants differ in their ability to induce in vitro transformation. Further studies aimed at elucidating potential pathogenic differences and functional significance of HPV 16 variants are anticipated.

Identification of specific HPV variants in sex partners has been applied to transmission studies [43]. Correlations between the variants detected has provided evidence for the sexual transmission of HPVs. Similarly, studies of HPV persistence have utilized variant analysis to examine persistent versus newly acquired HPV infections [44]. Caution should be taken when implementing or evaluating such studies as it is clear that the significance of these measurements is dependent on the actual prevalence of particular HPV variants in the study population. If a single variant predominates in a population, the significance of detecting that variant is not high for purposes of marking persistence or transmission events. Furthermore, the genomic region targeted for variant analysis should be optimized to provide the maximum available information. Current data indicate that the N-terminus of the HPV 16 E6 coding region provides the greatest information in the shortest targeted segment [35].

Sequence diversity of HPVs within individual HPV types, the relationship of intra- and intertypic variation, and the potential significance of HPV variants and subtypes have been given little consideration as factors important for determining the distribution of papillomaviruses and the risk for developing HPV-associated disease. The relevance of specific HPV sequences to several factors including transmissibility, host factors, persistence and oncogenicity needs to be considered further.

The identification of a more persistent or oncogenic HPV 16 variant(s) would facilitate the development of improved HPV diagnostic tests, and such variants could be important to the design of therapeutics and vaccines. Data indicate that naturally occuring antibodies to the HPV major capsid protein react almost exclusively to conformational dependent epitopes [45, 46]. Therefore little is known about the specific amino acids involved in HPV antigenic recognition. Given the potential importance of HPV sequence variation, the prevalence of given variants in different populations may be relevant to the rational design of HPV vaccine strategies.

Part I of this compendium contains alignments representing the current available data on HPV variants. This information may be useful when considering the functional significance of these variants and as molecular epidemiologic correlates of HPV16-associated disease.

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