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Lack of heterogeneity of HPV16 E7 sequence compared with HPV31 and HPV73 may be related to its unique carcinogenic properties

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Abstract

To assess the role of human papillomavirus virus (HPV) genetics in cervical lesions, we sequenced the E7 gene of HPV16, 31, or 73 from singly infected women who (1) cleared the infection quickly, (2) had type-specific persistent infection, or (3) progressed to CIN2 or worse lesions. Four of the 296 HPV16 E7 nucleotides were variable, compared with 7 of 296 for HPV31 E7 and 4 of 296 for HPV73 E7. While most of the polymorphisms in HPV31 and -73 resulted in non-synonymous amino acid changes, the polymorphisms in the HPV16 E7 resulted in synonymous changes. The lack of heterogeneity of HPV16 E7 suggests high evolutionary purifying selection that might be related to the unique carcinogenicity of HPV16.

Introduction

Persistent infections with carcinogenic human papillomavirus (HPV) in a high-risk clade of the alpha genus cause virtually all cervical cancers [1,2]. There are 10–15 carcinogenic HPV genotypes; however, there are substantial differences even among closely related HPV types in their ability to induce malignant transformation.

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We are studying how HPV genetic variability contributes to HPV natural history, to gain insight into carcinogenic mechanisms using a biological system that is simpler than human genomic variability. This brief communication examines one of HPV's early genes, E7, which is an important oncoprotein because it abrogates the function of pRB and is persistently expressed in HPV-induced cancers [3].

In particular, we compared HPV16, 31, and 73, which are genetically closely related types that manifest vast differences in their natural histories and risks for cervical cancer. HPV 16 (alpha-9 species) is by far the most carcinogenic HPV type and the main target of HPV vaccines. It is also the most prevalent carcinogenic type and most likely to persist. It causes half of cervical cancers worldwide and an even higher fraction of extra-cervical HPV-induced cancers (e.g., anal and oropharyngeal) [2]. By comparison, HPV31 (another type in alpha-9 closely related to HPV16) [4] is less prevalent and much less carcinogenic. As an even more striking contrast, HPV73 (alpha-11, a closely related species), while relatively common, is only equivocally carcinogenic [1]. The role of viral genetics in producing these pathologic differences is largely unexplored.

Previous research has shown that the E7 gene of HPV16 is relatively stable, with few nucleotide changes [5–8], though there is not a lot of research comparing E7 of different HPV genotypes to each other. Our objective in this study was to identify genetic sequence differences in the E7 gene of HPV16, 31 and 73 that could explain the differences in natural history between types.

Methods

Study design

This study was a cross-sectional study nested within the Guanacaste Cohort, which is a large population-based study of HPV natural history in a cohort of ~10,000 women aged 18–99 in Costa Rica [9,10]. Cervical cells collected as part of the parent study (Guanacaste Cohort) were tested for the presence of the HPV genotype at each study visit. In addition, at each visit, a pelvic exam was conducted, and women with suspicious lesions on cytology were further evaluated by colposcopy and biopsy when indicated. We randomly selected 60 women with single HPV16 infection, 54 women with single HPV31 infection, and 20 women with single HPV73 infection who either (1) cleared the infection ($n = 20$ for HPV16, $n = 12$ for HPV31, and $n = 16$ for HPV73) within an average period of less than 1 year, (2) had persistent infection, i.e., the same HPV present at two consecutive visits at least 1 year apart ($n = 20$ for HPV16; $n = 22$ for HPV31; and $n = 4$ for HPV73), or (3) progressed to cervical intra-epithelial neoplasia grade 2 or more severe lesions (CIN2+) ($n = 20$ of HPV16; $n = 13$ for HPV31; none of the HPV73 progressed to CIN3+ in the Guanacaste Cohort).

HPV detection

Cervicovaginal samples obtained from participants were stored in standard transport medium until used for polymerase chain reaction based HPV DNA testing using the L1 MY09/11 consensus primers. Dot blot analysis was used for HPV typing as described previously [1,9].

E7 sequencing

A nested PCR was used to amplify the E7 open reading frame from the original clinical samples using type-specific primers (primer sequences available upon request). PCR-positive samples were sequenced using the inner PCR primer set used for the final round of amplification. The sequences were analyzed using Chromas 2 and compiled using SeqMan within the DNASTAR package. ClustalW as implemented within the BioEdit software package [11] was used to align the sequences to their respective prototypes downloaded from GenBank (HPV16 NC_001526;

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HPV31 NC_001527; HPV73 NC_006165). Contingency tables showing the association of each viral E7 single nucleotide polymorphism (SNP) by case status are presented.

Results

Table 1 shows a list of the samples available for this project. Samples from eight of the HPV16 samples, and two of the HPV73 samples did not yield amplification products.

Tables 2, 3, and 4 contain data on the nucleotide and amino acid changes observed in the E7 gene of HPV16, 31, and 73, respectively. Four nucleotide base changes (out of 296, 1.4%) were observed in HPV16 E7 compared with 7 changes in HPV31 E7 (7/296, 2.4%) and 4 (4/296, 1.4%) in HPV73 E7. These slight numerical differences are made striking by the following observation: all 4 base pair changes in HPV16 resulted in synonymous changes in the amino acid sequence of HPV16 E7, while most of the base pair changes in HPV31 and 73 resulted in non-synonymous variations, as shown in Tables 3 and 4. Thus, at the amino acid level, HPV16 was less variable than HPV31 or HPV73.

There was an insertion of six nucleotide base pairs in one instance in HPV31 E7, which was not observed in HPV16 or -73 E7.

Table 5 shows the results of the viral single nucleotide polymorphisms by case status. We did not observe any effect by case status, although the study was not able to detect differences less than an odds ratio of 5.

Conclusion

We observed a highly conserved E7 region for HPV16, but not for HPV31 and 73, especially at the level of amino acids, in agreement with previous research [5–8]. The few nucleotide variations in HPV16 E7 resulted in synonymous amino acid sequence changes, while most of the variations in HPV31 and 73 resulted in non-synonymous changes. Although we did not find the previously described HPV16 E7 nucleotide change in position 647 [5–8], three of the nucleotide changes we report were also reported by others and also resulted in synonymous amino acid changes [5–8]. We present two explanations for the lack of non-synonymous changes in HPV16 E7 in this study. First, the HPV16 E7 sequence represents the optimal solution for its function, and any mutants get outcompeted by the prototype. Second, the protein fold of E7 does not allow for mutations, suggesting that HPV16 E7 is at a position in the "fold space" where a single amino acid change unfolds the protein. Our study was not designed to differentiate between these possibilities. It is also possible that our findings were due to chance and hence should be corroborated in other settings.

This study had several unique characteristics. The multidisciplinary approach used coupled laboratory data with a rigorous epidemiologic study of a population-based cohort that is unique for its long and intensive follow-up. Even in this rigorous epidemiologic study, with careful selection of cases, we were unable to detect any associations in relation to case–control status, underscoring the need for further research in this area. This research complements the research community's ongoing efforts to identify HPV cofactors needed for malignant transformation by studying the role of viral genetic polymorphisms in cancer etiology and contributes to the overall understanding of HPV E7 protein interaction with host cellular factors in cervical cancer development. In summary, the lack of heterogeneity of HPV16 E7 suggests evolutionary purifying selection, which may be related to the unique carcinogenic properties of HPV16. Moreover, the E7 variations are unlikely to explain differences in pathologic potential in this study.

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Table 1

Study population by case status

	CIN2+	Persistent	Clearance/control	Total
HPV16	16	19	17	52
HPV31	13	22	12	47
HPV73	0	4	14	18
Total	29	45	43	117

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Table 2

Nucleotide and amino acid sequence variations in HPV16 E7

Nucleotide sequence				
Position relative to HPV16 whole genome[a]	612	732	789	795
Position relative to E7 gene	51	171	228	234
Reference nucleotide	A	T	T	T
Variation	G	C	C	G
Amino acid sequence				
Position relative to E7 gene	17	53	76	78
Reference amino acid	Pro	Phe	Ile	Thr
Variation	Pro	Phe	Ile	Thr

[a]We considered the first nucleotide of the GenBank submission the first nucleotide position

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Table 3

Nucleotide and amino acid sequence variations in HPV31 E7

Nucleotide sequence								
Position relative to HPV31 whole genome[a]	580	626	645	670	695	Insertion at 711	743	746
Position relative to E7 gene	21	67	86	111	136	152	184	186
Reference nucleotide	G	C	C	C	G	–	A	G
Variation	A	T	T	T	A	CATCCA	G	C
Amino acid sequence								
E7 position	7	23	29	37	46	–	63	63
Reference amino acid	Thr	His	Pro	Val	Glu	–	Lys	Lys
Variation	Thr	Tyr	Leu	Val	Lys	Thr, Ser, Asp	Glu	Asp

[a] We considered the first nucleotide of the GenBank submission the first nucleotide position

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Table 4

Nucleotide and amino acid sequence variations in HPV73 E7

Nucleotide sequence				
Position relative to HPV73 whole genome[a]	588	727	799	812
Position relative to E7 gene	92	168	240	253
Reference nucleotide	A	C	G	A
Variation	G	T	T	G
Amino acid sequence				
E7 Position	31	56	80	85
Reference amino acid	Asn	Asp	Glu	Thr
Variation	Ser	Asp	Asp	Ala

[a]We considered the first nucleotide of the GenBank submission the first nucleotide position

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Table 5

Mutations by case status

SNP*	CIN2+ *n* = 16 (%)	Persistent *n* = 19 (%)	Clearance/control *n* = 17 (%)
HPV16			
A51G	0	0	1 (5.9)
T171C	2 (12.5)	3 (15.8)	2 (11.8)
T228C	3 (18.8)	3 (15.8)	2 (11.8)
T234G	3 (18.8)	3 (15.8)	2 (11.8)
SNP	*n* = 13 (%)	*n* = 22 (%)	*n* = 12 (%)
HPV31			
G21A	6 (46.2)	9 (40.9)	2 (16.7)
C67T	1 (7.7)	3 (13.6)	4 (33.3)
C86T	0	2 (9.1)	0
C111T	3 (23.1)	15 (68.2)	6 (50.0)
G136A	3 (23.1)	14 (63.6)	6 (50.0)
A184G	13 (100.0)	22 (100.0)	12 (100.0)
G186C	0	1 (4.5)	2 (9.1)
SNP	*n* = 0	*n* = 4 (%)	*n* = 14 (%)
HPV73			
A92G	–	2 (50.0)	10 (71.4)
C168T	–	4 (100.0)	13 (92.9)
G240T	–	4 (100.0)	13 (92.9)
A253G	–	4 (100.0)	12 (85.7)

**SNP* single nucleotide polymorphism