Human Papillomavirus Subtypes Are Not Uncommon

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ABSTRACT

While both variants and types of human papillomavirus (HPV) are common, subtypes (2-10% sequence divergence in the L1 gene) have been considered to be rare. We searched GenBank and in-house databases using a 440 nt L1 fragment and identified 7, 30 and 10 subtypes/putative subtypes in the HPV genera *Alpha*, *Beta* and *Gamma,* respectively. The number of types/putative types in each genus was 54, 58 and 103. Thus, there appears to exist at least 47 different subtypes/putative subtypes of HPV and they seem to be particularly common in the genus *Beta*-papillomavirus.

Keywords: HPV, phylogeny, subtypes, cutaneous

INTRODUCTION

The papillomaviruses (PVs) are grouped into genera, species, types, subtypes and variants (de Villiers et al., 2004). Different genera share less than 60% nucleotide sequence similarity in the major capsid protein L1 open reading frame (ORF), while species share between 60% and 70% similarity. The L1 ORF of a PV type shares between 71% and 89% sequence similarity to the closest known type. Subtypes and variants share between 90-98%, and 98-99% sequence similarity, respectively, to any known PV type. The PVs have been detected in a number of mammals and birds (Antonsson et al., 2003a; Antonsson and Hansson, 2002). To date, about 100 human PVs (HPVs) have been cloned and completely sequenced (de Villiers et al., 2004). In addition, there appears to exist about a hundred additional HPV types with sequence information so far available only from PCR amplimers (putative types) (Antonsson et al., 2003a; Antonsson et al., 2000; Antonsson et al., 2003b; Forslund et al., 1999; Forslund et al., 2004). HPV variants are quite commonly found and appear to associate with ethnic groups and with biologic behaviour of the virus (Calleja-Macias et al., 2005; Xi et al., 1997). By contrast, subtypes are considered rare (Bernard, 2005; Bernard et al., 2006; de Villiers et al., 2004). It is well known that HPV variants within the same type constitute the same serotype (Pastrana et al., 2001) and that different HPV types usually constitute different serotypes (Dillner and Brown, 2004). However, it is in general not known whether HPV subtypes are serologically distinct or not, except for HPV5 (a member of the genus *Beta*) that has been reported to exist as serologically distinct subtypes (Favre et al., 2000). Except for HPV38b[FA125] (Hazard et al., 2006), the only completely characterised subtypes presented in the literature (de Villiers et al., 2004; Narechania et al., 2005; Reuter et al., 1991; Trujillo et al., 1996; Yamaguchi et al., 2005) and available from GenBank belong to the genus *Alpha*-papillomavirus. Although the reasons for the infrequent reporting of HPV subtypes are not

known, the issue of whether HPV subtypes is frequent or not has profound implications for design of virus detection systems and for understanding of the biology of HPV.

The broad PCR primers FAP 59/64 amplify most so far known papillomaviruses and generate an amplicon of about 480 nucleotides (primers included) in the L1 ORF (the FA amplicon) (Forslund et al., 1999). A large number of FA amplicons have been isolated, particularly in skin samples (Antonsson et al., 2003a; Antonsson et al., 2000; Antonsson and Hansson, 2002; Antonsson et al., 2003b; Forslund et al., 1999; Forslund et al., 2004). A majority of these FA amplicons represent putatively new HPV types, whereas others demonstrate sequence similarities between 90-98%, indicating possible subtypes (Antonsson et al., 2003a; Antonsson et al., 2000; Antonsson and Hansson, 2002; Antonsson et al., 2003b; Forslund et al., 1999; Forslund et al., 2004).

In this study, we first aimed to investigate whether the sequence information of the FA amplimers is valid for taxonomic purposes. If so, we wanted to address the issue of whether HPV subtypes are rare or not, using the sequence information available from FA amplicons.

RESULTS

The estimates of sequence similarity using the complete L1 ORF and the FA fragments were equivalent. The differences did not appear to be systematically larger or smaller: the mean value of the sequence similarity difference was 0.11% (ranging from -1.5 to 2.3%, SD 1.36) (Table 1). Eight out of nine subtypes were classified as subtypes also when using the sequence information of the FA fragments. HPV27b was misclassified as a variant (Table 1). Comparison with the sequences amplified by the degenerate primer pair MY09/11 (Manos, 1989), another of the most frequently used general primer systems that has about the same fragment length as the FA primer system found very similar results, including misclassifying HPV27b as a variant (Table 1). Almost all the sequence variability in HPV27b is found at the

very C-terminal end, resulting in a frame shift mutation with unusually long L1 protein (Yamaguchi et al., 2005). As this is likely to be an exceptional case, the use of the about 450 nt FA or MY fragments for phylogeny appears to in general produce robust results.

We concluded that estimation of sequence similarity based on FA amplicons, representing about 30% of the L1 gene, produced only minor classification errors and proceeded to search GenBank and our in-house database for sequences corresponding to the FA amplicon.

We found 103 HPV type/putative type isolates within the genus *Gamma*-papillomavirus. These isolates substantially outnumber the 54 and 58 type/putative type isolates that we found in the genera *Alpha*- and *Beta*-papillomaviruses, respectively. However, subtypes appeared to be more frequently detected in the genus *Beta*-papillomavirus where we identified 30 subtype/putative subtype isolates compared to 10 subtypes/putative subtypes in the genus *Gamma* and only seven subtype isolates among the *Alpha*-papillomaviruses (Table 2). Compared to the number of types/putative types, there were significantly more subtypes detected within the genus *Beta* (30 subtypes versus 58 types) than in the genus *Gamma*-papillomaviruses (10 subtypes versus 103 types) ($p>0.001$).

The amino acid identities between the nine HPV type-subtype pairs for the entire L1 protein was on average 96.5% (range 94.5-98.4%). The amino acid sequence diversity between HPV15 and FA161, between HPV57 and HPV57b, and between HPV38 and HPV38b clustered within the hyper-variable loops of L1, while the amino acid differences were more evenly distributed over the complete L1 gene for the other genotype-subtype pairs (data not shown).

DISCUSSION

We report that existence of HPV subtypes is relatively common and that subtypes are significantly more common in the *Beta* genus than in the *Gamma* genus. Since the FAP59/64

PCR system is usually used for skin samples, less information on FA amplicons among *Alpha*-papillomaviruses is expected as these viruses are usually found in mucosal rather than in cutaneous epithelium and are usually identified by other detection systems. It is therefore not appropriate to directly compare the *Alpha* genus with the *Beta* and *Gamma* genera containing cutaneous HPV types, regarding the number of subtypes detected in relation to the number of genotypes.

We have previously reported that different PCR systems designed to detect HPV38 vary greatly in their ability to detect HPV38b, which is an equally frequent infection in skin lesions and normal skin as is the HPV38 prototype (Hazard et al., 2006). Needless to say, clinical and epidemiological studies that do not take the subtype diversity into account when designing and validating detection systems may give misleading results. We previously noted that the sequence diversity between HPV38 and HPV38b clustered in regions of the L1 gene encoding surface-exposed loops and in the E4 protein, a target for cellular immunity to HPV (Hazard et al., 2006). In the present study we observed that the sequence diversity between HPV15 and FA161 and between HPV57 and HPV57b also clustered within the hyper-variable loops of L1, while this was not the case for the other genotype-subtype pairs. It is interesting to note that HPV38, HPV15 and HPV57 have a cutaneous tropism, whereas the viruses with L1 amino acid differences spread out over the L1 gene mostly have a mucosal tropism.

If HPV subtypes confer partial immunity to each other or are even immunologically distinct, this may have implications for the population dynamics and natural history of cutaneous HPV infections. Our findings that HPV subtypes are common would therefore seem to motivate further studies on the immunological relatedness of HPV subtypes.

In conclusion, subtypes appear to be more common than earlier anticipated, at least among HPV types with cutaneous tropism. Existence of these subtypes has profound implications for design of virus detection systems and for the understanding of the biology of the viruses.

MATERIALS AND METHODS

Eight HPV type-subtype pairs were identified by MedLine searches. One type-subtype pair was identified in an in-house sequence database. Alignments of each genotype-subtype were performed using ClustalW, both with sequence data from FA amplimers (approximately 440 nt of the L1 gene), sequence data from MY09/11 amplimers (approximately 409 nt of the L1 gene) and the complete L1 of each pair of genotype and subtype.

In a series of previous studies (Antonsson et al., 2003a; Forslund et al., 2004; unpublished observations), sequences of amplimers obtained with FA PCR have been compared to GenBank. If not corresponding to any known HPV type, they have been given a provisional designation (FA-number). Currently our database contains approximately 220 "FA" isolates. In the current study, these isolates were compared to all HPV sequences in GenBank and to each other and classified as putative new types, subtypes or variants depending on their sequence similarity in the FA fragment.

ACKNOWLEDGEMENTS

The study was financed by the Swedish Research Council K2003-06XD-14532-01A and the Cancer Foundation of the University Hospital, Malmö.

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Table 1. Comparison of the sequence similarity between the FA and MY fragments and the complete L1 open reading frame of nine HPV type/subtype pairs.

Type (Accession number)	Subtype (Accession number)	Sequence similarity			
		FA[a] fragment	MY[a] fragment	L1 open reading frame	Δ[b] (FA-L1)
HPV15 **(X74468)**	FA161[c] (Malmö HPV database)	96.8%	94.9%	95.7%	+1.1%
HPV27 **(74473)**	HPV27b **(AB211993)**	99.8%	99.5%	97.5%	+2.3%
HPV38 **(U31787)**	HPV38b[FA125] **(DQ090005)**	95.2%	95.9%	95.6%	-0.4%
HPV44 **(U31788)**	HPV55 **(U31791)**	91.7%	92.5%	93.2%	-1.5%
HPV54 **(NC_001676)**	HPV54A **(AF436129)**	95.2%	94.4%	95.4%	-0.2%
HPV57 **(X55965)**	HPV57b **(U37537)**	94.7%	96.8%	95.7%	-1.0%
HPV68a **(DQ080079)**	HPV68ME180 **(M73258)**	92.0%	92.5%	92.8%	-0.8%
HPV74 **(U40822)**	HPV74AE10 **(AF436130)**	94.0%	93.1%	94.5%	-0.5%
HPV82 **(AB027021)**	HPV82A **(AF293961)**	94.3%	90.1%	92.3%	+2.0%

[a] The FA fragment is located at nt 5785-6224 and the MY fragment at nt 6602-7013 in the HPV16 genome (NC_001526) (primers not included).

[b] Δ is calculated as the difference between the sequence similarity of the FA fragment and the complete L1 open reading frame between the HPV type and its subtype.

[c] The complete L1 ORF of the FA161 isolate is recently cloned and sequenced by us (unpublished observation).

Table 2. Sequence similarity between genotypes and subtypes of the genera *Alpha*-, *Beta*-, and *Gamma*-papillomaviruses based on FA amplicon sequences (440 bp L1 fragments).

Genus	Genotype/ Putative genotype	Subtype/ putative subtype	Sequence similarity
Alpha	HPV27 **(X74473)**	HPV27b **(AB211993)**	99.8% [a]
	HPV44 **(U31788)**	HPV55 **(U31791)**	91.7%
	HPV54 **(NC_001676)**	HPV54A **(AF436129)**	95.2%
	HPV57 **(X55965)**	HPV57b **(HPU37537)**	94.7%
	HPV68a **(DQ080079)**	HPV68-ME180 **(M73258)**	92.0%
	HPV74 **(HPU40822)**	HPV74-AE10 **(AF436130)**	94.0%
	HPV82 **(AB027021)**	HPV82A **(AF293961)**	94.3%
Beta	HPV5 **(NC_001531)**	FA146 (Malmö HPV database)	95.4%
	HPV15 **(X74468)**	FA161 (Malmö HPV database)	96.6%
	HPV17 **(X74469)**	FA140 **(AY502597)**	96.1%
	HPV20 **(U31778)**	FA129 **(AY468426)**	97.0%

	FA130 **(AY468427)**	95.5%
	FA124 **(AY468421)**	93.1%
HPV23 **(U31781)**	FA123 **(AY468420)**	97.5%
HPV24 **(NC_001683)**	FA122.1 **(AY468417)**	92.6%
	FA122.2 **(AY468418)**	96.0%
HPV25 **(X74471)**	FA141 **(AY502598)**	96.8%
HPV37 **(U31786)**	FA131 **(AY468428)**	97.7%
	FA131.2 (Malmö HPV database)	93.0%
HPV38 **(U31787)**	HPV38b[FA125] **(DQ090005)**	95.2%
HPV92 **(AF531420)**	FA56 **(AY040275)**	92.0%
HPV93 **(AY382778)**	FAIMVS6.2 **(AF489709)**	93.5%
	FAIMVS6.4 **(AF489711)**	92.8%
	FAIMVS6.5 **(AY468439)**	92.8%
HPV96	FA39	90.0%

	(AY382779)	**(AF217684)**	
		FA47	97.5%
		(AY009881)	
	FA7	FA162	94.1%
	(AF121429)	**(DQ418477)**	
		FAIMVS16	92.7%
		(AY170668)	
	FA16.1	FA16.3	94.3%
	(AF217658)	**(AY468441)**	
	FA18	FAIMVS11.1	90.8%
	(AF217661)	**(AF489716)**	
	FA22	FA52	91.1%
	(AF217665)	**(AY009883)**	
	FA23.1	FA23.3	96.1%
	(AF217666)	**(AY484509)**	
	FA26	FA26.2	93.5%
	(AF217671)	**(AY468442)**	
		FA26.3	94.2%
		(AY468443)	
	FA51	FA51.2	97.9%
	(AY009885)	**(AY468438)**	
	FA84	FA84.2	95.5%
	(AF479248)	**(AY484510)**	
	FA119	FA158	94.1%
	(AY468414)	**(DQ418474)**	
Gamma	HPV50	FA143	97.5%
	(NC_001691)	(Malmö HPV database)	

FA6 **(AF121428)**	FA6.2 **(AY468437)**	96.6%
FA12 **(AF121433)**	FA12.2 **(AY502596)**	95.7%
FA13 **(AF121434)**	FA13.2 (Malmö HPV database)	96.1%
FA20 **(AF217663)**	FA20.2 **(AY468434)**	92.3%
FA91 **(AY081198)**	FAIMVS15.1 **(AF489723)**	91.2%
	FAIMVS15.3 **(AY468440)**	90.1%
FA107.1 **(AY204690)**	FA107.2 **(AY204691)**	97.3%
FA115 **(AY468410)**	FA150 **(DQ418466)**	96.6%
FAIMVS8 **(AF489713)**	FA157 **(DQ418473)**	95.5%

[a]As shown in Table 1, this subtype was the only one where classification using FA amplicon sequence differed from that of the complete L1 ORF, where the sequence similarity was 97.5%.