Identification of HPV82-BA10, A New Variant of the Papillomavirus Type 82, in a Pre-Cancerous Cervical Lesion

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Abstract

Human Papillomaviruses (HPVs) account for 75% of invasive cervical cancers worldwide. Viral variants of these HPVs differ in evolutionary history and pathogenicity. The taxonomic status of HPV types, subtypes, and variants is based on the traditional criterion that the sequence of their L1 genes should be, at least, 10%, 2-10 %, and maximally 2 % dissimilar from other well known HPVs. Recently, the genome of a new human papillomavirus (HPV-82) was detected in a vaginal intraepithelial neoplasia grade I, thus classified in super group A, together with HPV-26, -51, and -69, probable high risk virus types in cervical cancer. In this report, we demonstrate the presence of a new Variant of HPV Type 82, named HPV82-BA10, in a patient with a pathological cervical lesion.

ABBREVIATIONS

PVs: Papillomaviruses; HPV: Human Papillomavirus; ICTV: International Committee on the Taxonomy of Viruses; ORF: open reading frame, L1: late1; E6: early6; E7: early7; H-SIL: high grade squamous intraepitelial lesion; L-SIL: low grade squamous intraepitelial lesion; L.E.E.P: Loop Electrosurgical Excision Procedure; PCR: polymerase chain reaction; HR-HPV: high risk human papillomavirus; LR-HPV: low risk human papillomavirus.

INTRODUCTION

More than 120 types of human papillomaviruses have been identified from clinical samples. Papillomaviruses (PVs) have a genome of closed-circular double stranded DNA [1]. These viruses are now officially recognized by the International Committee on the Taxonomy of Viruses (ICTV) as a Family, Papillomaviridae, separated from Papovaviridae. The genital human PVs are now considered as genus: “the Alpha-Papillomavirus genus”. The taxonomic status of PV types, subtypes, and variants remain unchanged and is based on the traditional criteria that the sequence of their L1 genes should be at least 10%, 2-10 %, and maximally 2 % dissimilar from another [2,3]. HPVs are associated with benign and malignant neoplasia of cutaneous and mucosal epithelia. In fact, infection with Alpha – HPVs is the main cause of cervical cancer [4]. HPV type 16 and 18 were first classified as high-risk (HR-HPV) in 1986 [5], and then eight other HPVs (31, 33, 35, 45, 51, 52, 56, and 58) were added in 1992 [6,7]. The genome of a novel human papillomavirus (HPV-82) was detected in a vaginal intraepithelial neoplasia grade I. In a series of 291 biopsy specimens, HPV-82 was identified in one case, each of cervical intraepithelial neoplasia grade II and grade III, by blot hybridization. The histological localization of HPV-82 DNA, in the three lesions, was confirmed by in situ hybridization. The results indicated that HPV-82 might be an etiologic agent of vaginal and cervical intraepithelial neoplasia grade I [8]. By nucleotide sequence similarity, of L1 open reading frame (ORF), HPV-82 was closely related to HPV-26, -51, and -69 [9]. In this report, we demonstrate the presence of a new variant of HPV Type 82, named HPV82-BA10, in a woman with a cervical lesion and a severe dysplasia.

CASE PRESENTATION

A 44 years old woman, nulliparous, entered the menopause in 2004, showed the precocity of the climateric symptoms after few months. In May 2004, she underwent to Papanicolau test (Pap test) of cervix and endocervical cells that revealed...
the presence of a severe intraepithelial lesion. The subsequent biopsy confirmed a high grade squamous intraepithelial lesion (H-SIL), and a low grade squamous intraepithelial lesion (L-SIL). The patient underwent cervical linked region asportation by L.E.E.P. (Loop Electrosurgical Excision Procedure), and, after 6 months, she subjected another one cervical cytological Pap test, in liquid phase (Thin Prep), negative for cancer. At the same time, a research of HPV DNA (Amplimedical S.P.A. Torino, Italy), on the cytological specimen in the liquid phase, resulted positive (Figure 1). In order to identify HPV genotypes of DNA sample, we used two methods: a) the procedure of AlphaStrip HPV kit (Alphagenics Diaco Biotechnologies, Trieste, Italy); b) ProDect® BCS HPV CHIP and ProDect® HPV TYPING (Bcs Biotech srl, Cagliari, Italy). In the first method, the test for HPV genotypes identification was based on amplification of a part of L1 viral region (450 bp) by polymerase-chain-reaction (PCR) using the primers MY09-MY10; while a shorter sequence (150 bp) was obtained by nested PCR, using the primers GP5 – GP6 [10]. This method involves genotyping strip of 25 HPV types (Figure 2). ProDect® BCS HPV CHIP, instead, allows to simultaneously detecting different types of Human Papillomavirus (HPV). It is based on Polymerase Chain Reaction (PCR) amplification and nucleic acid hybridization of the target sequences on a polymer chip. Specific segments of the L1 and E6/E7 genes of HPV are amplified and hybridized with type-specific target probes pre-spotted on the surface of the chip. The kit is capable of detecting and genotyping the following HPV types: HR-HPV types 16,18,31,33, 45, and 58; LR-HPV types 6,11,34,70. The sample was tested in parallel with some positive and negative control samples. Both methods (Figure 2) clearly demonstrate negative results for the HPV types analyzed. (Figure 3, 4). DNA was also subjected to hybridization with specific
experimental probes: 26, 53, 59, 68, 73, 82. The hybridization of DNA extracted from cervical swab, with these experimental probes, revealed that the sample tested was strongly positive for the probe 82 (Figure 5). Thus, we cloned the entire L1 ORF and the E6 and E7 genes for sequencing and we identified a new variant of HPV type 82, called HPV82-BA10 (Gene Bank Accession number: DQ403744, DQ403743, DQ403742). This strain contains 12 mutations in the L1 gene (Figure 6) compared to HPV type 82 (Figure 6), and only 2 mutations in E6 and E7, respectively. This virus HPV 82 variant appears to be a genotype, detected for the first time in a severe cervical lesion.

**DISCUSSION**

HPV is believed to be an important factor in the pathogenesis of infecting and transforming epithelium, to particular benign and malignant lesions in humans; approximately 120 HPV subtypes have been isolated from humans [11]. HR-HPV encodes a series of proteins, E6 and E7 oncoproteins, that have been associated with cell transformations, which lead to genomic instability that can result in malignancy [12,13]. HR-HPV infection has been thought to be the main cause of human cervical cancers, a substantial proportion of other anogenital cancers, and oropharyngeal cancers [14]. HPV infection is also reported to be associated with lung cancers [15], and HR-HPVs were found in pre-cancerous lesions and/or cancer prostate [16]. Recently, we identified a new variant of HPV82, called HPV82-BA10, in a patient with pathological cervical lesion. This new virus variant seems to be involved in cervical neoplasia, being detected in cervical intraepithelial lesions (H-SIL), before and after surgery [17]. Moreover, the results demonstrate the importance of sequencing analysis in order to determine any genotype of HPV in a biological sample. The analysis of HPV82-BA10 L1 ORF showed a nucleotide difference of about 0.8% compared with HPV82 and should, therefore, be considered an HPV82 variant or a variant sublineage, on the basis of a recent phylogenetic analysis [18]. Variant and subtype classification of HPV types, identified by oligonucleotide probe methods, may need to be refined, especially for less prevalent HPVs and when little information on HPV prevalence is available. HPV genotyping is feasible and economical as the first choice of HPV screening and enables detection and typing of all known and, as yet, unknown genital HPVs. Further and deeper analyses are necessary to understand the role and involvement of this HPV82 variant in the pathogenesis of cervical cancer.
Figure 6  Alignment of L1 sequence of HPV 82-BA10 (upper strand) and HPV 82 (lower strand)
Upper sequence HPV 82-BA10
Lower sequence HPV 82 (GeneBankBAA90742.1)
Mutations are labelled in purple
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REFERENCES