Gynecologic Oncology 126 (2012) 304–311

Contents lists available at SciVerse ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Review

Susceptibility to cervical cancer: An overview

Antonio Carlos de Freitas [a,b,*], Ana Pavla Almeida Diniz Gurgel [a,b], Bárbara Simas Chagas [a], Eliane Campos Coimbra [b], Carolina Maria Medeiros do Amaral [a]

[a] Laboratory of Molecular Studies and Experimental Therapy, Department of Genetics, Center for Biological Sciences, Federal University of Pernambuco, Recife, Brazil
[b] Post-Graduation Program of Therapeutic Innovation, Federal University of Pernambuco, Recife, Brazil

ARTICLE INFO

Article history:
Received 3 December 2011
Accepted 29 March 2012
Available online 4 April 2012

Keywords:
Human Papillomavirus (HPV)
Cervical cancer
Susceptibility to cervical cancer

ABSTRACT

Cervical cancer is the second most common cancer in females worldwide. It is well-established that Human Papillomavirus (HPV) infections play a critical role in the development of cervical cancer. However, a large number of women infected with oncogenic HPV types will never develop cervical cancer. Thus, there are several external environment and genetic factors involved in the progression of a precancerous lesion to invasive cancer. In this review article, we addressed possible susceptible phenotypes to cervical cancer, focusing on host genome and HPV DNA variability, multiple HPV infections, co-infection with other agents, circulating HPV DNA and lifestyle.

Contents

Introduction

At present, cervical cancer is the second leading cause of cancer worldwide [1]. In the past 30 years, many efforts have been made to explain its etiology. It is generally agreed that Human Papillomavirus (HPV) infection plays a decisive role in the development of cervical lesions [2]. HPV is a small, non-enveloped, double-stranded genome virus that can cause both benign and malignant lesions in epithelial tissues (skin and mucosa) [3]. As well as cervical cancer, HPV infection can cause anogenital and oropharyngeal cancers and has also been linked to breast, lung, prostate and colorectal cancers [2].

High-risk HPV infection is necessary but not sufficient to cause cervical cancer. This statement is corroborated by the fact that a large

* Corresponding author at: Departmente of Genetics, Federal University of Pernambuco, Cidade Universitária, CEP 50.670-901, Recife, Pernambuco, Brazil.
E-mail addresses: acf_ufpe@yahoo.com.br (A.C. de Freitas), apavla@yahoo.com.br (A.P.A.D. Gurgel), babisimas@gmail.com (B.S. Chagas), elianeccoimbra@gmail.com (E.C. Coimbra), carolinamed3@gmail.com (C.M.M. do Amaral).

0090-8258/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2012.03.047

number of the women infected with HPV will never develop this cervical disease, which means that other factors are involved (Fig. 1). Several studies have been carried out with the host genome and its HPV DNA variability, in an attempt to show that there is a significant relationship between a specific genotype and susceptibility to cervical cancer. Furthermore, other co-factors are involved in susceptibility to cervical cancer such as individual lifestyle, circulating HPV DNA, co-infection with multiple HPV types and co-infection with other agents.

In this article, a number of external factors that may increase the risk of developing cervical cancer are addressed. Initially, we outline the relevant epidemiological data, as well as the natural history of HPV infection. Additionally, we discuss the genes involved in host susceptibility to cervical cancer. Furthermore, we analyze the HPV DNA variability and its putative impact on tumorigenesis. Finally, we address some additional factors, apart from HPV infection, that may be involved in the development of cervical cancer.

Cervical cancer epidemiology

Cervical cancer represents 9% of cases of female cancer and is the third leading cause of cancer in women worldwide, with more than 529,000 new cases and 275,000 deaths per year [1]. 85% of the cervical cancer occurs in developing countries. The estimate of global cervical cancer prevalence is 11.7%, and is most prevalent in Sub-Saharan Africa (24.0%), Eastern Europe (21.4%), and Latin America (16.1%) [4]. The problem of morbidity and mortality caused by cervical cancer is different in developed countries from that of developing countries where there are inadequate cervical cancer prevention and control programs. Developed countries have reduced the number of cervical cancer cases by approximately 80% as a result of effective programs for the detection and treatment of precancerous lesions [5]. In contrast, in developing countries it is difficult to conduct clinical screening of precancerous lesions since their National Health Systems have limited financial resources. Currently, the prevention strategies for cervical cancer include papanicolaou (or pap-smear) analysis and excision of precancerous lesions. About 85% of cervical cancer is squamous cell cancer, followed by adenocarcinoma and small cell neuroendocrine tumor [6]. However, in the last few years an increase in the rates of adenocarcinoma has been observed, particularly among young women [7]. Although screening programs have reduced the number of cases, cervical cancer remains one of the major causes of death among women worldwide.

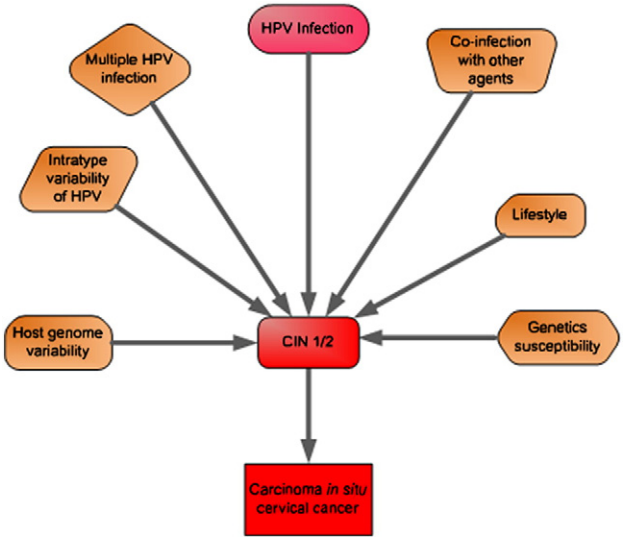

Fig. 1. Several genetics and environment factors involved in susceptibility to cervical cancer. It is well-established that HR-HPV is necessary but not sufficient to develop cervical cancer. Thus, other factors are involved in cervical cancer such as genetic susceptibility, host genome variability, HPV intratype variability, multiple HPV infections, co-infection with other agents and lifestyle.

HPV infection and cervical cancer

HPV infection

It is widely accepted that there is a link between cervical cancer and persistent infection caused by Human Papillomavirus (HPV) [2]. According to the International Committee on Taxonomy of Viruses (ICTV), HPV belongs to the *Papillomaviridae* family, which comprises 29 genera and 189 Papillomaviruses (PVs), including 120 Human PVs, 69 non-mammalian PVs, 3 PVs in birds and 2 PVs in reptiles [7]. All PVs share the same features including a non-enveloped virus and circular double-stranded DNA genome [8]. It is widely accepted that the HPV is specie-specific, epitheliotropic and mucosotropic, and usually infects keratinocytes [8], although recent investigations have found HPV DNA in non-epithelial sites such as blood [9,11–14], spermatozoa [15] and placenta [16].

To date, 120 HPV types have been described and these can be divided into five genera: *Alphapapillomavirus*, *Betapapillomavirus*, *Gammapapillomavirus*, *Mupapillomavirus* and *Nupapillomavirus* [3]. Approximately 40 of the HPV types can infect the genital tract. These types are classified according to the oncogenic potential in High-Risk (HR) HPVs: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82; and Low-Risk (LR) HPVs: 6, 11, 42, 44, 51, 53 and 83 [17]. The most common HR-HPV types worldwide are 16 (57%), 18 (16%), 58, 33, 45, 31, 52, 35, 59, 39, 51 and 56 [18]. On the other hand, approximately 90% of the LR-HPV infections (involved in benign skin lesions such as warts) are caused by the HPV-6 and HPV-11 types. Because of their medical importance, several studies have focused on HPV, since this virus group is involved in anogenital and non-anogenital cancers such as cervical, penis, vagina, vulva, anus, head–neck and non-melanoma skin cancers [5,19]. Furthermore, HPV infection occurs in benign diseases, such as anogenital warts, laryngeal papilomas, and psoriasis [5,19].

HPV is a non-enveloped, double-stranded, circular DNA virus, approximately 8 kb in size. HPV DNA has eight open reading frames (ORFs), namely E1, E2, E4, E5, E6, E7 (expressed in the early phase of infection), L1 and L2 (expressed in the late phase of infection) and control region designated as the long control region (LCR) [17,20] (Fig. 2). E1 and E2 ORFs are involved in viral DNA replication, although a recent study demonstrated that there was viral replication of HPV DNA without E1 or E2 ORFs [21]. The E5 ORF seems to induce the loss of surface MHC-I expression in the epithelial cells, leading to evasion of immune surveillance in the early stage of infection [22]. E6 and E7 ORFs are oncoproteins involved with proliferation-stimulating and transforming activities through the loss of E1 and E2 ORFs, allowing the integration of E6 and E7 ORFs within the host DNA. Once integrated, HPV DNA can immortalize human keratinocytes due to the interactions between the E6 and E7 oncogenes with p53 and pRb tumor suppressor proteins, respectively, thus inhibiting the process of apoptosis. Moreover, HPV DNA possesses a LCR, which regulates the transcription of the E6 and E7 viral oncogenes through the transcription factors of the virus and the host cells [17].

The natural history of cervical cancer caused by HPV was extensively revised in other works [2,17,20]. Briefly, as the result of a breach in the stratified epithelial tissue, the HPV can infect the basal cell layer and begin the process of infection. Host cell entry of HPV is initiated by binding the virus particles to the cell surface receptors, such as heparan sulfate. Subsequently, there is an expression of the E1, E2, E4, E5, E6 and E7 ORFs, thus resulting in the replication of the HPV DNA as an episome. The infected host cell divides and spreads out laterally, causing infection of the suprabasal cell layers. In this phase, L1 and L2 ORFs are expressed resulting in viral capsid formation. Finally,

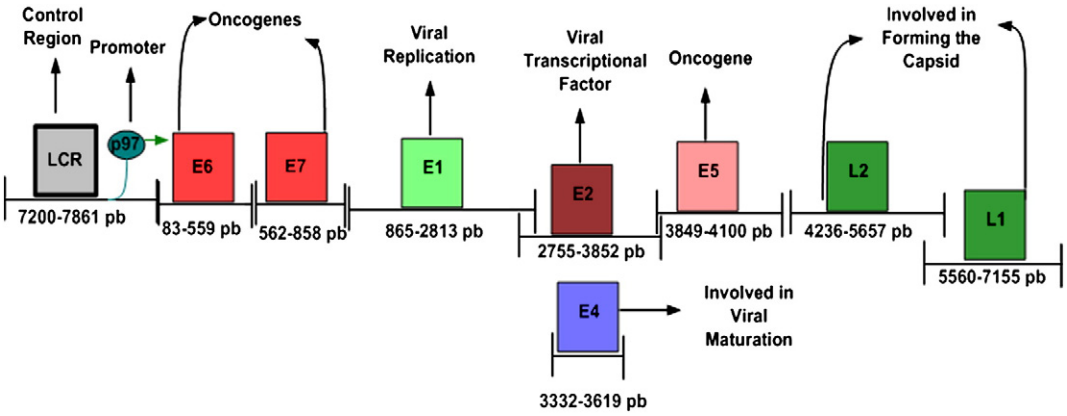

Fig. 2. HPV-16 complete genome is shown in a linear format (based in RefSeq NC_001526.2). After the entry of the HPV into the basal cell layer begins the expression of the E1, E2, E4, E5, E6 and E7 ORFs, thus resulting in the replication of the HPV DNA as an episome. In the last stage of the infection, the L1 and L2 ORFs are expressed. Several studies suggest that HPV DNA variability could be involved in the persistence and progression to cervical cancer. The LCR region regulates the transcription of the E6 and the E7 oncogene through the p97 promoter. Studies have shown that polymorphisms in LCR region can increase the p97 promoter activity more than three-fold. Furthermore, polimorphic sites in E6 and E7 ORFs could result in oncoprotein, which may be involved in an efficient inactivation of p53 and pRb proteins, respectively. The E2 ORF is a viral transcription factor that regulates the expression of E6 and E7 oncogenes, thus polymorfic sites in E2 ORF could alter the binding of the E2 transcription factor. Polymorphism in L1 ORF could alter: i) the structure of the capsid protein; ii) the immune recognition; and iii) the viral neutralization, thus interfering in vaccine strategies.

the HPV virion is released at the cell surface, which can result in infection of other sites.

HR-HPV infection is commonly transmitted by sexual intercourse and can cause lesions in epithelial tissues, which can regress for 6–12 months [17]. It is not clearly understood why HPV infections resolve in certain cases and result in premalignant lesions that can progress to cervical cancer in others. Individual susceptibility and other enabling factors may play a relevant role [23]. In this context, activation of the host's immune system seems to have a central role in the resolution of HPV infection [24]. There is estimation that 70% of HPV infections are resolved spontaneously within 1 year [25] and about 90% resolve within 2 years [26]; reflecting that immune response to HPV infection is generally slow and weak [27]. This fact is supported by a variety of mechanisms adopted by the virus for evading immunological detection. The first of these is that it is a non-lytic virus (does not cause the death of infected cell) because the release of the viral particles occurs through the programmed death of keratinocytes (desquamation). Thus, there is no viremia and the essential signals for the immune response in epithelium as the production of proinflammatory cytokines that activate the migration of antigen-presenting cells (APCs), are absent [47,48]. Apart from the fact that the cycle of the HPV is practically invisible to the host [28,29] its oncoproteins have developed molecular mechanisms to facilitate the virus evasion of the immune system, such as: interference with interferon via anti-viral defense [30,31], reduction in the number of Langerhan cells-LCs (APCs of the epidermis) [43–45] ,inhibition of the expression of MHC-I complex [32] and the change in expression of toll-like receptor 9 (TLR-9), which has an essential role in pathogen recognition and activation innate immunity [33]. However, non-treatment of these lesions can allow premalignant conditions to progress to cervical cancer. These cervical lesions can be characterized and detected by cytological and histopathological clinical examinations. If women are not treated, these premalignant condition can progress to: cervical intraepithelial neoplasia grade 1 (CIN1) (or mild dysplasia); intraepithelial neoplasia grade 2 (CIN2) (or moderate dysplasia); intraepithelial neoplasia grade 3 (CIN3) or *in situ* carcinoma, characterized by severe dysplasia; and squamous cell carcinoma or adenocarcinoma [17]. In the Bethesda System, other terms are employed such as: atypical squamous cells of undetermined significance (ASCUS); squamous intraepithelial lesions (SIL), which comprises the low-grade SIL (mild dysplasia) and high-grade SIL (moderate severe dysplasia and *in situ* carcinoma) [34]. About 28% of the cervical intraepithelial neoplasia grade 2/3 leads to complete regression in a short period of time; however, lesions associated with HPV-16 are less likely to undergo regression [35]. In fact, in clinical practice, it is not possible to distinguish between lesions that are likely to regress from those that are not. Thus, these lesions are usually treated surgically, to prevent a progression to cervical cancer.

HPV infection in non-epithelial tissue

Although HPV infects epithelial tissues, several studies have shown that circulating HPV DNA is present in non-epithelial tissues. In this context, HPV DNA was found in plasma [9,12,13,36–38], peripheral blood [10,11,39] and sera [14,40] of patients with cervical intraepithelial neoplasia or cervical cancer, making the circulating HPV DNA as a possible marker for cervical disease. The exact pathway through which the viral tumor-derived DNA is released into the peripheral blood is still unknown. In addition, a study has shown that there is a perinatal transmission of HPV [41]. Furthermore, circulating HPV DNA have been found in non-epithelial sites such as spermatozoa [15], prostatic tissue [42] placenta [16], and in several other diseases including esophageal [43,44], breast [45], colorectal [46] and lung cancers [47]. Moreover, HPV DNA was found in newborns [41] and pediatric patients without any history of sexual intercourse [39]. The range of this evidence leads us to speculate that the HPV is spread through a hematogenic pathway.

Susceptibility to cervical cancer

Genetic epidemiological evidences

The etiology of cervical cancer can be fully explained in terms of the infections caused by oncogenic HPV types, as outlined above. However, although infections caused by HR-HPV are a necessary feature, they are not sufficient in themselves to develop cervical cancer. It is widely reported that few of the women who are infected with oncogenic HPV types will develop cervical cancer. This question remains unresolved. It is likely that genetic and environmental factors are involved in susceptibility to cervical cancer, such as host genetic variability, intratype variations of HPV, co-infection with multiple HPV types, co-infection with other agents and lifestyle (Fig. 1).

HPV infection depends on interactions between the host cell and virus genome, which can make an individual susceptible to cervical cancer. Genetic–epidemiological studies about the heritability of cervical cancer have shown a familial aggregation of cervical intraepithelial neoplasia and cervical cancer in first-degree relatives [48–51]. Moreover, the evidence of genetic inheritance and susceptibility to cervical cancer were supported in twin studies that investigated smear abnormalities [48] and cervical cancer [52]. In addition, susceptibility to cervical cancer was observed in other phenotypes, such as Fanconi anemia patients. In this group of patients, there is an increase in the risk of developing cervical cancer and vulvar cancer [28,53–55] when compared with patients that do not suffer from Fanconi anemia. Thus, these genetic epidemiological studies strongly suggest that host genetics play a role in susceptibility to cervical disease. However, the genes involved in this process are still unknown.

Association studies with candidate gene

It is well-known that down-regulation of the tumor suppressor genes may be involved in host susceptibility to cervical cancer. In recent years, several association studies have been conducted with genes involved in cellular cycle and apoptosis induction, such as *TP53*, *MDM2*, *CDKN2A* and *CDKN1A* and their putative role in cervical cancer in women infected with HPV [56–65]. The *TP53* gene codifies the p53 tumor suppressor protein involved in apoptosis process. The *TP53* gene codifies the p53 tumor suppressor protein involved in apoptosis process. The *TP53* gene is considered to be a putative candidate gene in cervical cancer since the E6-AP complex is able to target p53 for degradation via the E6AP ubitiquin ligase. Thus, polymorphism in the *TP53* gene may be better targeted for degradation by the E6-AP complex. Several studies have been conducted to find a link between codon P72R polymorphism of p53 and the risk of developing cervical cancer. Some studies have found that P72R polymorphism of p53 is associated with cervical [62,65] and adenocarcinoma cancers [59], however, other investigations have failed to replicate this data [56,62,63].

Other genes, besides *TP53* are involved in susceptibility to cervical cancer. *MDM2* gene encodes the human homolog of mouse double minute 2, a nuclear phospholipoprotein that inhibits p53 protein. Thus, polymorphism in the *MDM2* gene could be a candidate for susceptibility to cervical cancer. A study carried out by Nunobiki et al. [66] showed that there was a significant link between T309G polymorphism and cervical cancer [66], although these results have not been corroborated by other investigations [58,65,67]. *WAF1* also known as cyclin-dependent kinase inhibitor 1 (*CDKN1A*) or CDK-interacting protein 1, encode the tumor suppressor protein p21. *CDKN1A* gene acts as an inhibitor of cyclin-dependent kinases (Cdks) and p53 regulates the expression of p21 protein. Single-nucleotide polymorphisms in *CDKN1A* codon Arg31Ser seem to be associated with cervical cancer [59,68]. In addition to the p53 pathway, the genes involved in the human leukocyte antigen system (HLA) also play a role in cervical cancer susceptibility. It was reported that polymorphisms in the HLA system are associated with cervical cancer [69,70]. As discussed above, there is some divergence in association studies concerning the effects of these genes on susceptibility to cervical cancer. With regard to this question, a recent study has shown that this disagreement is due to errors arising from the types of methodologies employed [64]. Thus, it is necessary to use alternative methodologies in association studies with candidate genes, such as gene expression studies. In view of this , the Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) which codifies the tumor suppressor protein p16 gene, is overexpressed in HPV positive patients when compared to those who are HPV negative, due to the inactivation of pRb by E7 oncoprotein [71–74]. Taken together, the evidence strongly suggests that host genes are involved in making women infected with HPV susceptible to cervical cancer (Table 1).

Table 1
Genetics association studies involving p53 pathway in susceptibility to cervical cancer.

Gene	Polymorphism	Reference
TP53	P72R	[56,58,61–64].
MDM2	T309G	[57,64–66]
CDKN2A	Arg31Ser	[58,67]

Lifestyle and co-infection in cervical cancer

Individual lifestyle is also likely to have an effect on susceptibility to cervical cancer. Studies have shown an association between tobacco smoke and cervical cancer due to the reduction of the immune response and the carcinogenic effects of tobacco [75–79]. Moreover, the use of oral contraceptives can also lead to cervical cancer since there is an increase in the expression of HPV genes [80]. Furthermore, early sexual activity [81] and multiple sex partners [81,82] are cofactors that are independently associated with abnormal cytology and cervical cancer.

Women who have multiple HPV types of infection might be more susceptible to develop cervical cancer than those who have only one HPV type, since these viruses can act synergistically. In the light of this, a recent study showed that there was an association between multiple HPV types and cervical intraepithelial neoplasia and cervical cancer [83,84], although the same results were not found in other populations [85]. In addition, co-infection with other agents has also been found in several studies. An association between HR-HPV and *Chlamydia trachomatis* (CT) has been shown to incur a risk of cervical intraepithelial neoplasia and abnormal cytology when compared with patients without CT infection [82,86].

A relationship between Human immunodeficiency virus (HIV) infection and invasive cervical cancer was established in several studies. The HIV positive patients are susceptible to cervical cancer and cervical intraepithelial neoplasia due to the HIV induced immunosuppression, and both HIV and HPV interact synergically [87]. Thus, multiple infections with HR-HPV as well as infection with other agents, such as HIV and CT, seem to play a critical role in furthering the progression to cervical intraepithelial neoplasia and cervical cancer.

HPV DNA variability

There is a considerable amount of data showing that the intratype sequence variation of HPV is involved in the persistence and progression to cervical cancer [83,88–93]. These intratype variants may differ in their biological and etiological aspects, and affect the oncogenic potential for the development of cervical cancer [88]. The differences in nucleotide sequences of HPV can result in changes in encoded amino acids, which may alter the oncogenic potential. Furthermore, these differences in HPV DNA sequences may result in disparities in the incidence of cervical cancer worldwide [90].

Studies have demonstrated the existence of differential biological behavior of HPV variants, for example, Asian-American variants are commonly found in young patients with cervical cancer and in severe lesions [94], while other variants have been associated with specific histological features [95].

A new PVs is recognized when the L1 ORF nucleotide sequence differs by more than 10% from all the described types. In addition, HPV DNA is classified as a molecular variant when the nucleotide sequence has a similarity of 98% when compared to a prototype sequence [3,96]. However, the nucleotide variability among the molecular variants can be as high as 5% in the non-coding region (LCR) [96].

Studies concerned with genetic variability revealed that different variants of HPV-16 and HPV-18 co-evolved with the three major human phylogenetic branches: Africans, Caucasians and Asians. However, variants of HPV-16 were grouped into five distinct groups spread in different geographical regions, such as Europe (E), Asia (As),

Asian-America (AA), Africa 1 (AF-1) and Africa 2 (Af-2) [10,97]. The variants of HPV-18 were grouped into three distinct groups: European (E), Asian-American (AA) and African (Af) [98–100]. With regard to HPV-31, a study carried out by Chagas et al. [83] suggested that HPV-31 variants do not display the above-mentioned patterns of co-evolution with human ethnic groups. The same inconsistency was replicated in other study [101].

Intratype HPV-16 sequence variation

With regard to HPV-16, several investigations found an association between non-European variants and a higher risk of developing cervical intraepithelial neoplasia or cervical cancer than European variants [88,89,96]. These studies are concentrated on LCR and E6 and E7 ORFs. However, other investigations have also found variability within the L1 and E2 ORFs. Intratype sequence variations in the L1 gene can play an important role in the structure of the capsid protein, immune recognition, viral neutralization and interference in vaccine strategies [88]. In this sense, it was demonstrated that a variation Asp202His in the L1 protein can assemble into virus-like particles (VLPs) more efficiently than its prototype L1 [102]. In addition, it was found that variations in the 83–97 residues of the L1 gene have an effect on the yield of the L1 protein [103]. Despite these findings, some studies have not found any association between the variability in the L1 gene and cervical intraepithelial neoplasia or cervical cancer [104,105].

Nucleotide variations in E6 and E7 ORFs play a critical role in the development of cervical cancer due to their ability to inactivate p53 and pRb proteins, respectively [106]. For this reason, several studies have been conducted of the viral variants of the HPV-16 E6 ORF and risk of cervical neoplasia. The appearance of invasive tumors from high grade precursor lesions has been associated with the accumulation of variants of E6. Epidemiological studies have shown association between the progression of high grade precursor lesions to invasive tumors and the accumulation of an HPV-16 variant harboring a T to G transition at nucleotide 350 of the E6 oncogene corresponding to amino acid L83V [107–110]. In addition, a variation of the A276G E6 ORF increases the risk of developing cervical intraepithelial neoplasia and cervical cancer [111,112]. In the Japanese and Chinese populations was reported a substitution at position 25 (D25E) of the E6 protein also associated with the progression of cervical carcinoma ([111,113]. Previous study showed that HPV-16 E6 D25E is the most prevalent variant in Korean women at high risk for developing cervical cancer [114]. With regard to the E7 gene, it was found that the variability at position 647 of the HPV-16 E7 gene of HPV-16 was more frequent in cervical cancer than the precursor lesions [115]. Among the oncogenes E6 and E7 of HPV-16, E6 shows more variation than E7, which is relatively considered conserved [97,108,116,117].

Apart from the E6 and E7 ORFs, some studies have examined the variability of E2 ORF in cervical cancer. In non-transformed cells, E2 protein regulates the transcription of the E6 and E7 oncoproteins. Thus, variability in the E2 ORF can potentially alter the expression of the E6 and E7 oncogenes. A study carried out by Giannoudis et al. [118] found that there was a significant link between the C3684A variant of HPV-16 and cervical intraepithelial neoplasia. However, other study on E2 variants has not provided evidence of an association with cervical cancer [119].

The LCR is the binding site of cellular and viral transcription factors. These transcriptional factors possess an ability to activate or suppress the p97 promoter, and regulate the HPV-16 E6 and E7 expression. Thus, variability in the nucleotide sequence of the binding site of these transcriptional factors can alter the expressions of the E6 and E7 oncogenes. In this context, the G7521A variation has been found in several studies of cervical cancer [93104104,109,120,121]. The G7521A variant is located in the YY1 binding site and it was demonstrated that this variation can increase the p97 promoter activity three to six-fold [122] (Table 2).

Table 2
Studies concerning HPV DNA variability most often found in cervical intraepithelial neoplasia and cervical cancer.

HPV	ORFs/Regions	References
HPV-16	*L1*, *E2*, *E6*, and LCR	[87,88,92,95,96,101–111,110,112–116,118–121].
HPV-18	*E6*, *E7* and LCR	[122–128,130].
HPV-31	*E6*, *E7* and LCR	[82,100,131–133,135,133,125].
HPV-33 e 58	*E6* and *E7*	[125,133,135,133].

Intratype HPV-18 sequence variation

It has been suggested that genomic variability of different HPV-18 isolates might be responsible for the wide spectrum of pathologies associated with this viral type. It was identified an HPV-18 variant absent in cervical cancer, but present in 40% of intraepithelial lesions, suggesting a lower oncogenic potential [123].

Studies have found a nucleotide change of C491A in HPV-18 E6 ORF [124,125], although *in vitro* analysis has shown that this E6 ORF variation does not affect its ability to cause p53 degradation [126]. Variations in the HPV-18 E6 ORF were also observed at positions 287, 485 and 549, in the form of silent mutations [127]. Moreover, there were variations in the HPV-18 E7 gene located near a linear epitope, which is present on the surface of the capsid and is common among many HPV genotypes [128,129]. Cerqueira et al. [125] found A41G and T104C variations within HPV-18 LCR. These variations seem to be able to achieve a increase activity of the E6/E7 p97 promoter by modulating Sp1 and YY1 activities [130]. The studies that found specific HPV-18 variants were associated with cervical cancer in different populations [89,93,96,131] (Table 2).

Intratype HPV-31 sequence variation

As regards the HPV-31 E6 gene, Chagas et al. [83] detected variations at positions 213 and 413, and similar results were found in other studies [101,132]. Furthermore, Chagas et al. [83] found several variants in HPV-31 E6 and E7 genes located in T-cell and B-cell epitope sites [83]. These variations may influence the display of viral peptides in the T-cells. The recognition of T-cell determinants by T-cells (particularly T helper cells) significantly strengthens the cell-mediated immune response against infectious organisms. T cells cooperate with B cells in the induction and maintenance of an effective antibody response and this leads to the maturation of cytotoxic T cells by interacting with macrophages [133]. Apart from HPV-31 E6 and E7 genes, studies have been conducted with HPV-31 LCR. Recently, it was shown that the A6943C and T6949A variability were associated with high grade cervical lesions [134]. Analysis of HPV-31 LCR revealed the G7449A, G7457A, C7474T, G7525A and T7575C variations, which potentially affected the binding sites for the transcription factors [134] (Table 2).

Intratype HPV-33 and HPV-58 sequence variation

HPV-33 E6 gene variations were associated with 71% of the cases of cervical intraepithelial neoplasia [135]. Regarding the HPV-33 E7 gene, two nucleotide changes A737G and A862T were detected, but only one displayed a change in codon (A862T, Q97L) [136]. There have still been hardly any studies on HPV-33 genomic variability and only a few HPV-33 genomic variants have been described (Table 2).

HPV-58 LCR sequence variation showed that C to G transversion at the 7284 was found in 21.7% of abnormal cervical cytology patients [134]. Furthermore, a significant association was found between T7207A,

C7284G, T7345C, T7369G, T431G and T7483G and abnormal cervical cytology [125] (Table 2).

Thus, these studies suggest that the HPV DNA variability may be associated with cervical intraepithelial neoplasia and cervical cancer (Fig. 2). HPV DNA variability might also explain the geographical differences in morbidity and mortality worldwide.

Conclusions and perspectives

This article has discussed several critical issues involving the question of susceptibility to cervical cancer. It is well-known that HPV infection is necessary but not sufficient to develop cervical cancer, which suggests that there are external factors involved in this process. Genetic epidemiological studies suggest that genes are involved in the susceptibility to cervical cancer, although these genes associated with this process are still unknown. Thus, there is a need for other association studies, which can clarify] the role of host genes in susceptibility to cervical cancer. Furthermore, circulating HPV DNA infects several non-genital tissues, which suggests the hematogenous spread of HPV DNA leading to the persistence, progression and pathogenesis of cervical cancer. Moreover, factors related to an individual lifestyle, such as the use of tobacco, hormonal contraceptives, early sexual activity and multiple sex partners are also associated with cervical cancer. Multiple HPV infections and co-infection with other agents also seem to play a critical role in susceptibility to cervical cancer. HPV DNA variability has been associated with persistence and progression of a premalignant condition to cervical cancer; however, to date, there is no consensus about the molecular markers that reveal susceptibility to cervical cancer. Taken together, this evidence can explain why many women have HPV infection, but only a few of them develop cervical cancer. Thus, clinical follow-up of cervical intraepithelial neoplasia patients which takes account of the critical areas discussed in this work, could clarify the role played by genetic and environmental factors in the progression of cervical intraepithelial neoplasia to cervical cancer.

Conflict of interest statement
The authors declare that there are no conflicts of interest.

Acknowledgments

We would like to express our thanks to André Luís Jesus and Fillipe Colaço and Fernando Matos for reviewing this paper.

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. J Clin 2011;61:69–90.
[2] zur Hausen H. Papillomavirus infections-a major cause of human cancers. Biochim Biophys Acta 1996;1288(2):F55–78.
[3] de Villiers E-M, Fauquet C, Broker TR, Bernard H-U, zur Hausen H. Classification of Papillomaviruses. Virology 2004;324(1):17–27.
[4] Bruni L, Diaz M, Castellsagué M, Ferrer E, Bosch FX, de Sanjosé S. Cervical Human Papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis 2010;202(12):1789–99.
[5] Hoory T, Monie A, Gravitt P, Wu T-C. Molecular epidemiology of Human Papillomavirus. J Formos Med Assoc 2008;107(3):198–217.
[6] Bray F, Carstensen B, Møller H, Zappa M, Žakelj MP, Lawrence G, et al. Incidence trends of adenocarcinoma of the cervix in 13 European countries. Cancer Epidemiol Biomarkers Prev 2005;14(9):2191–9.
[7] Bernard HU, Burk RD, Chen Z, van Doorslaer K, Hausen H, de Villiers EM. Classification of Papillomavirus (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 2010;401(1):70–9.
[8] Bravo IG, de Sanjosé S, Gottschling M. The clinical importance of understanding the evolution of Papillomaviruses. Trends Microbiol 2010;18:432–8.
[9] Gnanamony M, Peedicayil A, Subhashini J, Ram TS, Rajasekar A, Gravitt P, et al. Detection and quantitation of HPV 16 and 18 in plasma of Indian women with cervical cancer. Gynecol Oncol 2010;116(3):447–51.
[10] Ho C-M, Yang S-S, Chien T-Y, Huang S-H, Jeng C-J, Chang S-F. Detection and quantitation of Human Papillomavirus type 16, 18 and 52 DNA in the peripheral blood of cervical cancer patients. Gynecol Oncol 2005;99(3):615–21.
[11] Kay P, Allan B, Denny L, Hoffman M, Williamson A-L. Detection of HPV 16 and HPV 18 DNA in the blood of patients with cervical cancer. J Med Virol 2005;75(3):435–9.
[12] Sathish N, Abraham P, Peedicayil A, Sridharan G, John S, Shaji RV, et al. HPV DNA in plasma of patients with cervical carcinoma. J Clin Virol 2004;31(3):204–9.
[13] Pornthanakasem W, Shotelersuk K, Termrungruanglert W, Voravud N, Niruthisard S, Mutirangura A. Human Papillomavirus DNA in plasma of patients with cervical cancer. BMC Cancer 2001;1(1):2.
[14] Liu VW, Tsang P, Yip A, Ng TY, Wong LC, Ngan HY. Low incidence of HPV DNA in sera of pretreatment cervical cancer patients. Gynecol Oncol 2001;82(2):269–72.
[15] Kaspersen MD, Larsen PB, Ingerslev HJ, Fedder J, Petersen GB, Bonde J, et al. Identification of multiple HPV types on spermatozoa from human sperm donors. PLoS One 2011;6(3):e18095.
[16] Weyn C, Thomas D, Jani J, Guizani M, Donner C, Van Rysselberge M, et al. Evidence of Human Papillomavirus in the placenta. J Infect Dis 2011;203(3):341–3.
[17] zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002;2(5):342–50.
[18] Li N, Franceschi S, Howell-Jones R, Snijders PJF, Clifford GM. Human Papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication. Int J Cancer 2011;128(4):927–35.
[19] Pfister H. Chapter 8: Human Papillomavirus and skin cancer. J Natl Cancer Inst Monogr 2003;31:52–6.
[20] Doorbar J. The Papillomavirus life cycle. J Clin Virol 2005;32:7–15.
[21] Pittayakhajonwut D, Angeletti PC. Viral trans-factor independent replication of Human Papillomavirus genomes. Virol J 2010;7:123.
[22] Venuti A, Paolini F, Nasir L, Corteggio A, Roperto S, Campo MS, et al. Papillomavirus E5: the smallest oncoprotein with many functions. Mol Cancer 2011;10(1):140.
[23] Steben M, Duarte-Franco E. Human Papillomavirus infection: epidemiology and pathophysiology. Gynecol Oncol 2007;107(2 Suppl 1):S2–5.
[24] Frazer I. Correlating immunity with protection for HPV infection. Int J Infect Dis 2007;11(Suppl. 2):S10–6.
[25] Zur Hausen H. Papillomaviruses in human cancers. Proc Assoc Am Physicians 1999;111(6):581–7.
[26] Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal Papillomavirus infection in young women. N Engl J Med 1998;338(7):423–8.
[27] Frazer IH. Prevention of cervical cancer through Papillomavirus vaccination. Nat Rev Immunol 2004;4(1):46–54.
[28] Lowy DR, Gillison ML. A new link between fanconi anemia and Human Papillomavirus-associated malignancies. J Natl Cancer Inst 2003;95(22):1648–50.
[29] Stanley M, Gissmann L, Nardelli-Haefliger D. Immunobiology of Human Papillomavirus infection and vaccination—implications for second generation vaccines. Vaccine 2008;26(Supplement 10(0):K62–7.
[30] Ronco LV, Karpova AY, Vidal M, Howley PM. Human Papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. Genes Dev 1998;12(13):2061–72.
[31] Barnard P, McMillan NA. The Human Papillomavirus E7 oncoprotein abrogates signaling mediated by interferon-alpha. Virology 1999;259(2):305–13.
[32] Bottley G, Watherston OG, Hiew Y-L, Norrild B, Cook GP, Blair GE. High-risk human Papillomavirus E7 expression reduces cell-surface MHC class I molecules and increases susceptibility to natural killer cells. Oncogene 2007;27(12):1794–9.
[33] Hasan UA, Bates E, Takeshita F, Biliato A, Accardi R, Bouvard V, et al. TLR9 expression and function is abolished by the cervical cancer-associated Human Papillomavirus type 16. J Immunol 2007;178(5):3186–97.
[34] Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The 2001 Bethesda system. JAMA 2002;287(16):2114–9.
[35] Trimble CL, Piantadosi S, Gravitt P, Ronnett B, Pizer E, Elko A, et al. The Johns Hopkins Medical Institutions B. Spontaneous regression of high-grade cervical dysplasia: effects of Human Papillomavirus type and HLA phenotype. Clin Cancer Res 2005;11(13):4717–23.
[36] Wei Y-C, Chou Y-S, Chu T-Y. Detection and typing of minimal Human Papillomavirus DNA in plasma. Int J Gynaecol Obstet 2007;96(2):112–6.
[37] Shimada T, Yamaguchi N, Nishida N, Yamasaki K, Miura K, Katamine S, et al. Human Papillomavirus DNA in plasma of patients with HPV16 DNA-positive uterine cervical cancer. Jpn J Clin Oncol 2010;40(5):420–4.
[38] Dong SM, Pai SI, Rha S-H, Hildesheim A, Kurman RJ, Schwartz PE, et al. Detection and quantitation of Human Papillomavirus DNA in the plasma of patients with cervical carcinoma. Cancer Epidemiol Biomarkers Prev 2002;11(1):3–6.
[39] Bodaghi S, Wood LV, Roby G, Ryder C, Steinberg SM, Zheng Z-M. Could Human Papillomaviruses be spread through blood? J Clin Microbiol 2005;43(11):5428–34.
[40] Widschwendter A, Blassnig A, Wiedemair A, MÃller-Holzner E, MÃller HM, Marth C. Human Papillomavirus DNA in sera of cervical cancer patients as tumor marker. Cancer Lett 2003;202(2):231–9.
[41] Rombaldi RL, Serafini EP, Mandelli J, Zimmermann E, Losquiavo KP. Perinatal transmission of Human Papilomavirus DNA. Virol J 2009;6:83.
[42] Zambrano A, Kalantari M, Simoneau A, Jensen JL, Villarreal LP. Detection of Human Polyomaviruses and Papillomaviruses in prostatic tissue reveals the prostate as a habitat for multiple viral infections. Prostate 2002;53(4):263–76.
[43] Shen Z-Y, Hu S-P, Lu L-C, Tang C-Z, Kuang Z-S, Zhong S-P, et al. Detection of Human Papillomavirus in esophageal carcinoma. J Med Virol 2002;68(3):412–6.

[44] Gábor B, Imdahl A, György L, Pál O. HPV-infection in esophageal cancer as possible predictive factor after neoadjuvant therapy. Magy Seb 2006;59(2): 97–104.
[45] Widschwendter A, Brunhuber T, Wiedemair A, Mueller-Holzner E, Marth C. Detection of Human Papillomavirus DNA in breast cancer of patients with cervical cancer history. J Clin Virol 2004;31(4):292–7.
[46] Bodaghi S, Yamanegi K, Xiao S-Y, Da Costa M, Palefsky JM, Zheng Z-M. Colorectal Papillomavirus infection in patients with colorectal cancer. Clin Cancer Res 2005;11(8):2862–7.
[47] Klein F, Amin Kotb WFM, Petersen I. Incidence of Human Papilloma virus in lung cancer. Lung Cancer 2009;65(1):13–8.
[48] Vink JM, van Kemenade FJ, Meijer CJLM, Casparie MK, Meijer GA, Boomsma DI. Cervix smear abnormalities: linking pathology data in female twins, their mothers and sisters. Eur J Hum Genet 2011;19(1):108–11.
[49] Hemminki K, Chen B. Familial risks for cervical tumors in full and half siblings: etiologic apportioning. Cancer Epidemiol Biomarkers Prev 2006;15(7):1413–4.
[50] Magnusson PKE, Lichtenstein P, Gyllensten UB. Heritability of cervical tumours. Int J Cancer 2000;88(5):698–701.
[51] Couto E, Hemminki K. Heritable and environmental components in cervical tumors. Int J Cancer 2006;119(11):2699–701.
[52] Ahlbom A, Lichtenstein P, Malmström H, Feychting M, Pedersen NL, Hemminki K. Cancer in twins: genetic and nongenetic familial risk factors. J Natl Cancer Inst 1997;89(4):287–93.
[53] Alter BP. Cancer in fanconi anemia, 1927–2001. Cancer 2003;97(2):425–40.
[54] Park JW, Pitot HC, Strati K, Spardy N, Duensing S, Grompe M, et al. Deficiencies in the Fanconi anemia DNA damage response pathway increase sensitivity to HPV-associated head and neck cancer. Cancer Res 2010;70(23):9959–68.
[55] Kutler DI, Wreesmann VB, Goberdhan A, Ben-Porat L, Satagopan J, Ngai I, et al. Human Papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. J Natl Cancer Inst 2003;95(22):1718–21.
[56] Hu W, Feng Z, Ma L, Wagner J, Rice JJ, Stolovitzky G, et al. A single nucleotide polymorphism in the MDM2 gene disrupts the oscillation of p53 and MDM2 levels in cells. Cancer Res 2007;67(6):2757–65.
[57] Bhattacharya P, Sengupta S. Lack of evidence that proline homozygosity at codon 72 of p53 and rare arginine allele at codon 31 of p21, jointly mediate cervical cancer susceptibility among Indian women. Gynecol Oncol 2005;99(1):176–82.
[58] Ueda M, Yamamoto M, Nunobiki O, Toji E, Sato N, Izuma S, et al. Murine double-minute 2 homolog single nucleotide polymorphism 309 and the risk of gynecologic cancer. Hum Cell 2009;22(2):49–54.
[59] Roh J-W, Kim BK, Lee CH, Kim J, Chung HH, Kim JW, et al. P53 codon 72 and p21 codon 31 polymorphisms and susceptibility to cervical adenocarcinoma in Korean women. Oncol Res Featuring Preclinical Clin Cancer Ther 2010;18(9): 453–9.
[60] Roh J-W, Kim BK, Lee CH, Kim J, Chung HH, Kim JW, et al. P53 codon 72 and p21 codon 31 polymorphisms and susceptibility to cervical adenocarcinoma in Korean women. Oncol Res 2010;18(9):453–9.
[61] Harima Y, Sawada S, Nagata K, Sougawa M, Ostapenko V, Ohnishi T. Polymorphism of the waf1 gene is related to susceptibility to cervical cancer in Japanese women. Int J Mol Med 2001;7(3):261–4.
[62] Jiang P, Liu J, Li W, Zeng X, Tang J. Role of p53 and p21 polymorphisms in the risk of cervical cancer among Chinese women. Acta Biochim Biophys Sin (Shanghai) 2010;42(9):671–6.
[63] Oliveira S, Sousa H, Santos AM, Pinto D, Pinto-Correia AL, Fontoura D, et al. The p53 r72p polymorphism does not influence cervical cancer development in a Portuguese population: a study in exfoliated cervical cells. J Med Virol 2008;80(3):424–9.
[64] Klug SJ, Ressing M, Koenig J, Abba MC, Agorastos T, Brenna SM, et al. Tp53 codon 72 polymorphism and cervical cancer: a pooled analysis of individual data from 49 studies. Lancet Oncol 2009;10(8):772–84.
[65] Hu X, Zhang Z, Ma D, Huettner PC, Massad LS, Nguyen L, et al. Tp53, MDM2, NQO1, and susceptibility to cervical cancer. Cancer Epidemiol Biomarkers Prev 2010;19(3):755–61.
[66] Nunobiki O, Ueda M, Yamamoto M, Toji E, Sato N, Izuma S, et al. MDM2 SNP 309 human papillomavirus infection in cervical carcinogenesis. Gynecol Oncol 2010;118(3):258–61.
[67] de Vasconcellos Meissner R, Niskier Ferreira Barbosa R, VerÃ-ssimo Fernandes J, Medeiros GalvÃ£o T, Oliveira Galvão AF, de Medeiros Oliveira GH. No association between SNP309 promoter polymorphism in the MDM2 and cervical cancer in a study from northeastern Brazil. Cancer Detect Prev 2007;31(5):371–4.
[68] Tian Q, Lu W, Chen H, Ye F, Xie X. The nonsynonymous single-nucleotide polymorphisms in codon 31 of p21 gene and the susceptibility to cervical cancer in Chinese women. Int J Gynecol Cancer 2009;19:1011–4.
[69] Chan PKS, Cheung T-H, Lin CK, Siu S-SN, Yim S-F, Lo KWK, et al. Association between HLA-DRB1 polymorphism, high-risk HPV infection and cervical neoplasia in southern Chinese. J Med Virol 2007;79(7):970–6.
[70] Hildesheim A, Schiffman M, Scott DR, Marti D, Kissner T, Sherman ME, et al. Human leukocyte antigen class I/II alleles and development of human papillomavirus-related cervical neoplasia: results from a case–control study conducted in the United States. Cancer Epidemiol Biomarkers Prev 1998;7(11): 1035–41.
[71] Kanao H, Enomoto T, Ueda Y, Fujita M, Nakashima R, Ueno Y, et al. Correlation between p14 and p16 expression and HPV infection in uterine cervical cancer. Cancer Lett 2004;213(1):31–7.
[72] Sano T, Oyama T, Kashiwabara K, Fukuda T, Nakajima T. Expression status of p16 protein is associated with Human Papillomavirus oncogenic potential in cervical and genital lesions. Am J Pathol 1998;153(6):1741–8.
[73] Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U, et al. Over-expression of p16 as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. Int J Cancer 2001;92(2):276–84.
[74] Volgareva G, Zavalishina L, Andreeva Y, Frank G, Krutikova E, Golovina D, et al. Protein p16 as a marker of dysplastic and neoplastic alterations in cervical epithelial cells. BMC Cancer 2004;4(1):58.
[75] Trimble CL, Genkinger JM, Burke AE, Hoffman SC, Helzlsouer KJ, Diener-West M, et al. Active and passive cigarette smoking and the risk of cervical neoplasia. Obstet Gynecol 2005;105(1):174–81.
[76] Moktar A, Singh R, Vadhanam MV, Ravoori S, Lillard JW, Gairola CG, et al. Cigarette smoke condensate-induced oxidative DNA damage and its removal in human cervical cancer cells. Int J Oncol 2011;39(4):941–7.
[77] MacLaughlan SD, Lachance JA, Gjelsvik A. Correlation between smoking status and cervical cancer screening: a cross-sectional study. J Low Genit Tract Dis 2011;15(2):114–9.
[78] Ward KK, Berenson AB, Breitkopf CR. Passive smoke exposure and abnormal cervical cytology in a predominantly Hispanic population. Am J Obstet Gynecol 2011;204(3):e1–6 213.
[79] Ma YT, Collins SI, Young LS, Murray PG, Woodman CBJ. Smoking initiation is followed by the early acquisition of epigenetic change in cervical epithelium: a longitudinal study. Br J Cancer 2011;104(9):1500–4.
[80] Ghanem KG, Datta SD, Unger ER, Hagensee M, Shlay JC, Kerndt P, et al. The association of current hormonal contraceptive use with type-specific HPV detection. Sex Transm Infect 2011;87(5):385–8.
[81] Au WW. Life style, environmental and genetic susceptibility to cervical cancer. Toxicology 2004;198(1–3):117–20.
[82] Finan RR, Tamim H, Almawi WY. Identification of chlamydia trachomatis DNA in human papillomavirus (HPV) positive women with normal and abnormal cytology. Arch Gynecol Obstet 2002;266(3):168–71.
[83] Baldez da Silva MFPT, Chagas BS, Guimarães V, Katz LMC, Felix PM, Miranda PM, et al. HPV31 and HPV33 incidence in cervical samples from women in Recife, Brazil. Genet Mol Res 2009;8(4):1437–43.
[84] Soto-De Leon S, Camargo M, Sanchez R, Munoz M, Perez-Prados A, Purroy A, et al. Distribution patterns of infection with multiple types of Human Papillomaviruses and their association with risk factors. PLoS One 2011;6(2):e14705.
[85] Chaturvedi AK, Katki HA, Hildesheim A, Rodríguez AC, Quint W, Schiffman M, et al. Human Papillomavirus infection with multiple types: pattern of coinfection and risk of cervical disease. J Infect Dis 2011;203(7):910–20.
[86] Safaeian M, Quint K, Schiffman M, Rodriguez AC, Wacholder S, Herrero R, et al. *Chlamydia trachomatis* and risk of prevalent and incident cervical premalignancy in a population-based cohort. J Natl Cancer Inst 2010;102(23):1794–804.
[87] Cejtin HE. Gynecologic issues in the HIV-infected woman. Infect Dis Clin North Am 2008;22(4):709–39 [vii].
[88] Sichero L, Villa LL. Epidemiological and functional implications of molecular variants of human papillomavirus. Braz J Med Biol Res 2006;39:707–17.
[89] Sichero L, Ferreira S, Trottier H, Duarte-Franco E, Ferenczy A, Franco EL, et al. High grade cervical lesions are caused preferentially by non-European variants of HPVs 16 and 18. Int J Cancer 2007;120(8):1763–8.
[90] Calleja-Macias IE, Kalantari M, Huh J, Ortiz-Lopez R, Rojas-Martinez A, Gonzalez-Guerrero JF, et al. Genomic diversity of Human Papillomavirus-16, 18, 31, and 35 isolates in a Mexican population and relationship to European, African, and native American variants. Virology 2004;319(2):315–23.
[91] Junes-Gill K, Sichero L, Maciag PC, Mello W, Noronha V, Villa LL. Human papillomavirus type 16 variants in cervical cancer from an admixtured population in Brazil. J Med Virol 2008;80(9):1639–45.
[92] López-Revilla R, Pineda MA, Ortiz-Valdez J, Sánchez-Garza M, Riego L. Human Papillomavirus type 16 variants in cervical intraepithelial neoplasia and invasive carcinoma in San Luis Potosí City, Mexico. Infect Agents Cancer 2009;4:3.
[93] Villa LL, Sichero L, Rahal P, Caballero O, Ferenczy A, Rohan T, et al. Molecular variants of Human Papillomavirus types 16 and 18 preferentially associated with cervical neoplasia. J Gen Virol 2000;81(Pt 12):2959–68.
[94] del Refugio González-Losa M, Laviada Mier y Teran MA, Puerto-Solís M, García-Carrancá A. Molecular variants of HPV type 16 e6 among Mexican women with LSIL and invasive cancer. J Clin Virol 2004;29(2):95–8.
[95] Berumen J. Nuevos virus del papiloma humano descubiertos en México y su asociación a la alta incidencia del cáncer de cérvix. Gac Med Mex 2003;139: S3–9.
[96] Bernard H-U, Calleja-Macias IE, Dunn ST. Genome variation of Human Papillomavirus types: phylogenetic and medical implications. Int J Cancer 2006;118(5):1071–6.
[97] Yamada T, Manos MM, Peto J, Greer CE, Munoz N, Bosch FX, et al. Human Papillomavirus type 16 sequence variation in cervical cancers: a worldwide perspective. J Virol 1997;71(3):2463–72.
[98] Ong CK, Chan SY, Campo MS, Fujinaga K, Mavromara-Nazos P, Labropoulou V, et al. Evolution of Human Papillomavirus type 18: an ancient phylogenetic root in Africa and intratype diversity reflect coevolution with human ethnic groups. J Virol 1993;67(11):6424–31.
[99] Arias-Pulido H, Peyton CL, Torrez-Martínez N, Anderson DN, Wheeler CM. Human Papillomavirus type 18 variant lineages in United States populations characterized by sequence analysis of LCR-E6, E2, and L1 regions. Virology 2005;338(1):22–34.
[100] Bernard H-U. The clinical importance of the nomenclature, evolution and taxonomy of Human Papillomaviruses. J Clin Virol 2005;32(Suppl. 1):S1–6.
[101] Raiol T, Wyant PS, de Amorim RMS, Cerqueira DM, Milanezi N von G, Brígido M de M, et al. Genetic variability and phylogeny of the high-risk HPV-31, -33, -35, -52, and -58 in Central Brazil. J Med Virol 2009;81(4):685–92.

[102] Kirnbauer R, Taub J, Greenstone H, Roden R, Durst M, Gissmann L, et al. Efficient self-assembly of Human Papillomavirus type 16 L1 and L1- into virus-like particles. J Virol 1993;67(12):6929–36.
[103] Touze A, El Mehdaoui S, Sizaret P-Y, Mougin C, Munoz N, Coursaget P. The L1 major capsid protein of human papillomavirus type 16-variants affects yield of virus-like particles produced in an insect cell expression system. J Clin Microbiol 1998;36(7):2046–51.
[104] Pande S, Jain N, Prusty BK, Bhambhani S, Gupta S, Sharma R, et al. Human Papillomavirus type 16 variant analysis of E6, E7, and L1 genes and long control region in biopsy samples from cervical cancer patients in north India. J Clin Microbiol 2008;46(3):1060–6.
[105] Wu Y, Chen Y, Li L, Yu G, He Y, Zhang Y. Analysis of mutations in the E6/E7 oncogenes and L1 gene of human papillomavirus 16 cervical cancer isolates from China. J Gen Virol 2006;87(5):1181–8.
[106] Kast W, Brandt R, Sidney J, Drijfhout J, Kubo R, Grey H, et al. Role of HLA motifs in identification of potential CTL epitopes in human papillomavirus type 16 E6 and E7 proteins. J Immunol 1994;152(8):3904–12.
[107] Londesborough P, Ho L, Terry G, Cuzick J, Wheeler C, Singer A. Human Papillomavirus genotype as a predictor of persistence and development of high-grade lesions in women with minor cervical abnormalities. Int J Cancer 1996;69(5):364–8.
[108] Zehbe I, Wilander E, Delius H, Tommasino M. Human Papillomavirus 16 E6 variants are more prevalent in invasive cervical carcinoma than the prototype. Cancer Res 1998;58(4):829–33.
[109] Kämmer C, Tommasino M, Syrjänen S, Delius H, Hebling U, Warthorst U, et al. Variants of the long control region and the E6 oncogene in European Human Papillomavirus type 16 isolates: implications for cervical disease. Br J Cancer 2002;86(2):269–73.
[110] Zehbe I, Lichtig H, Westerback A, Lambert PF, Tommasino M, Sherman L. Rare human papillomavirus 16 E6 variants reveal significant oncogenic potential. Mol Cancer 2011;10:77.
[111] Matsumoto K, Yoshikawa H, Nakagawa S, Tang X, Yasugi T, Kawana K, et al. Enhanced oncogenicity of human papillomavirus type 16 (HPV16) variants in Japanese population. Cancer Lett 2000;156(2):159–65.
[112] Hu X, Pang T, Guo Z, Mazurenko N, Kisseljov F, Pontén J, et al. HPV16 E6 gene variations in invasive cervical squamous cell carcinoma and cancer in situ from Russian patients. Br J Cancer 2001;84(6):791–5.
[113] Cai HB, Chen CC, Ding XH. Human Papillomavirus type 16 E6 gene variations in Chinese population. Eur J Surg Oncol 2010;36(2):160–3.
[114] Choi B-S, Kim SS, Yun H, Jang DH, Lee J-S. Distinctive distribution of HPV16 E6 D25E and E7 N29S intratypic Asian variants in Korean commercial sex workers. J Med Virol 2007;79(4):426–30.
[115] Song S-H, Lee J-K, Seok O-S, Saw H-S. The relationship between cytokines and HPV-16, HPV-16 E6, E7, and high-risk HPV viral load in the uterine cervix. Gynecol Oncol 2007;104(3):732–8.
[116] Tornesello ML, Duraturo ML, Salatiello I, Buonaguro L, Losito S, Botti G, et al. Analysis of Human Papillomavirus type-16 variants in Italian women with cervical intraepithelial neoplasia and cervical cancer. J Med Virol 2004;74(1):117–26.
[117] van Duin M, Snijders PJ, Vossen MT, Klaassen E, Voorhorst F, Verheijen RH, et al. Analysis of Human Papillomavirus type 16 E6 variants in relation to p53 codon 72 polymorphism genotypes in cervical carcinogenesis. J Gen Virol 2000;81(Pt 2):317–25.
[118] Giannoudis A, van Duin M, Snijders PJF, Herrington CS. Variation in the E2-binding domain of HPV 16 is associated with high-grade squamous intraepithelial lesions of the cervix. Br J Cancer 2001;84(8):1058–63.
[119] Bhattacharjee B, Sengupta S. Hpv16 E2 gene disruption and polymorphisms of E2 and LCR: some significant associations with cervical cancer in Indian women. Gynecol Oncol 2006;100(2):372–8.
[120] Shang Q, Wang Y, Fang Y, Wei L, Chen S, Sun Y, et al. Human Papillomavirus type 16 variant analysis of E6, E7, and L1 genes and long control region in identification of cervical carcinomas in patients in Northeast China. J Clin Microbiol 2011;49(7):2656–63.
[121] Schmidt M, Kedzia W, Goździcka-Józefiak A. Intratype HPV16 sequence variation within LCR of isolates from asymptomatic carriers and cervical cancers. J Clin Virol 2001;23(1–2):65–77.
[122] Dong XP, Pfister H. Overlapping YY1- and aberrant SP1-binding sites proximal to the early promoter of human papillomavirus type 16. J Gen Virol 1999;80(Pt 8):2097–101.
[123] Hecht JL, Kadish AS, Jiang G, Burk RD. Genetic characterization of the Human Papillomavirus (HPV) 18 E2 gene in clinical specimens suggests the presence of a subtype with decreased oncogenic potential. Int J Cancer 1995;60(3):369–76.
[124] De Boer MA, Peters LAW, Aziz MF, Siregar B, Cornain S, Vrede MA, et al. Human Papillomavirus type 18 variants: histopathology and E6/E7 polymorphisms in three countries. Int J Cancer 2005;114(3):422–5.
[125] Cerqueira DM, Raiol T, Véras NMC, Gal Milanezi N, Amaral FA, Macedo Brígido M, et al. New variants of Human Papillomavirus type 18 identified in Central Brazil. Virus Genes 2008;37:282–7.
[126] De la Cruz-Hernández E, García-Carrancá A, Mohar-Betancourt A, Dueñas-González A, Contreras-Paredes A, Pérez-Cardenas E, et al. Differential splicing of E6 within Human Papillomavirus type 18 variants and functional consequences. J Gen Virol 2005;86(9):2459–68.
[127] Halpen A, McBride A, Myers G, Baker C, Wheeler CM, Doobar J. Human papillomaviruses. a compilation and analysis of nucleic acid and amino acid sequences. Los Alamos, NM: Los Alamos National laboratory; 1997.
[128] Cerqueira DM, Raiol T, Véras NMC, Von Gal Milanezi N, Amaral FA, De Macedo Brígido M, et al. New variants of human papillomavirus type 18 identified in Central Brazil. Virus Genes 2008;37(2):282–7.
[129] Combita A-L, Touze A, Bousarghin L, Christensen ND, Coursaget P. Identification of two cross-neutralizing linear epitopes within the L1 major capsid protein of Human Papillomaviruses. J Virol 2002;76(13):6480–6.
[130] Rose BR, Thompson CH, Zhang J, Stoeter M, Stephen A, Pfister H, et al. Sequence variation in the upstream regulatory region of HPV 18 isolates from cervical cancers. Gynecol Oncol 1997;66(2):282–9.
[131] de Boer MA, Peters LAW, Aziz MF, Siregar B, Cornain S, Vrede MA, et al. Human Papillomavirus type 16 E6, E7, and L1 variants in cervical cancer in Indonesia, Suriname, and the Netherlands. Gynecol Oncol 2004;94(2):488–94.
[132] Calleja-Macias IE, Villa LL, Prado JC, Kalantari M, Allan B, Williamson A-L, et al. Worldwide genomic diversity of the high-risk Human Papillomavirus types 31, 35, 52, and 58, four close relatives of human papillomavirus type 16. J Virol 2005;79(21):13630–40.
[133] Comerford SA, McCance DJ, Dougan G, Tite JP. Identification of T- and B-cell epitopes of the E7 protein of Human Papillomavirus type 16. J Virol 1991;65(9):4681–90.
[134] Cento V, Rahmatalla N, Ciccozzi M, Perno CF, Ciotti M. Intratype variations of HPV 31 and 58 in Italian women with abnormal cervical cytology. J Med Virol 2011;83(10):1752–61.
[135] Xin CY, Matsumoto K, Yoshikawa H, Yasugi T, Onda T, Nakagawa S, et al. Analysis of E6 variants of Human Papillomavirus type 33, 52 and 58 in Japanese women with cervical intraepithelial neoplasia/cervical cancer in relation to their oncogenic potential. Cancer Lett 2001;170(1):19–24.
[136] Vrtačnik Bokal E, Kocjan BJ, Poljak M, Bogovac Ž, Jančar N. Genomic variants of Human Papillomavirus genotypes 16, 18, and 33 in women with cervical cancer in Slovenia. J Obstet Gynaecol Res 2010;36(6):1204–13.