Even if the global mortality has diminished in the last years, this cancer still kills more than 200,000 patients every year, accounting for more than 7% of all female cancer deaths, mostly in developing countries [1,2]. Oncogenic human papillomaviruses (HPVs) are the primary etiologic agents of cervical cancer, which occurs usually in the transformation zone (TZ) of the cervix [3]. Viral HPV genome is detected in almost all cases of cervical cancer with HPV types 16 and 18 causing 70% of them [5]. Persistent infections with one or more of the 15 oncogenic HPVs lead to the development of well-defined preneoplastic lesions or squamous intraepithelial lesions (SILs). Although HPV infections are frequent, most infected patients will clear the virus naturally within two years and more than 80% of the low grade intraepithelial lesions can regress spontaneously. Unfortunately HPV has developed several immunoescape mechanisms allowing persistent infection to progress into cervical neoplasia [6].

Mucosal oncogenic HPVs

To date, 174 HPV types are characterized based on the isolation of complete genomes [7,8] They are organized into five major HPV genera – Alpha-, Beta-, Gamma-, Mu-, and Nu-papillomavirus.
The *Alphapapillomavirus* genus contains all HPVs for which sufficient evidence qualifies them as oncogenic for humans [9]. They are also classified as cutaneous or mucosal according to which tissues are infected [7]. This review is focused on mucosal oncogenic HPV, which are the causative agents of uterine cervical cancers and are also etiologically associated with other anogenital tumors and head and neck carcinomas [10].

In 2009, an International Agency for Research on Cancer (IARC) Working Group classified 12 oncogenic mucosal HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) also named high-risk HPV. Twelve additional types (HPV 26, 30, 34, 53, 66, 67, 68, 69, 70, 73, 82, 85 and 97) were classified as probably carcinogenic [11]. Other mucosal HPV types in the *Alphapapillomavirus* genus (e.g. HPV6 and 11) are responsible for genital warts and benign infections and are referred as low-risk HPVs [4].

The most prevalent HPV type in the uterine cervix (normal exocervix and every grade of cervical lesions including cancer) is HPV16 [7]. In fact, HPV16 is detected in 52 to 58% of cervical cancers in the world and the second most prevalent HPV type in cancer is HPV18 (ranging from 13 to 22%) [12]. A HPV persistent infection is necessary for the development of cancer, but other cofactors are associated with the malignant transformation of cells infected by HPV such as immunosuppression [13], tobacco smoking [14] or sex hormones [15].

### Origin of HPV-induced uterine cervical lesions

**Cellular origin of cervix cancer**

Histologically, two types of epithelia are present in normal cervix. The endocervical canal is protected by a monolayer of glandular epithelium, which produces mucus, a barrier against pathogens, whereas the exterior of the cervix is covered, like the vagina, by a squamous epithelium. After puberty, a physiological process of squamous metaplasia leads to replacement of a portion of the endocervical epithelium by squamous epithelium. This region of metaplasia is named the transformation zone or TZ. Most of the uterine cervical cancers are squamous cell carcinoma (SCC). In a minority of cases, cervical adenocarcinoma (AC), which develops from the mucus-producing gland cells of the endocervix, is observed.

Determination of the target cell of HPV infection leading to cervical SCC and AC is still not well defined. Since there are two subtypes of cervical cancer, SCC and AC, it was proposed that SCC appears from squamous epithelium whereas AC derives from glandular epithelium [16]. However, some evidences, like the fact that some SCC started in the endocervix, suggested that SCC develops as a result of proliferation of the reserve or basal cells located into the endocervical canal [17]. Those findings have led to advances in the diagnosis of cervix cancer, particularly for intracervical curettage and Pap smears test [18]. Study of Burghardt and Ostor revealed that almost all cervical preneoplastic lesions arise from the TZ, thereby defining more precisely the anatomical origin of cervical cancer [3]. Later, based on keratin expression shared by several carcinomas, it was proposed that this expression pattern reflects a common progenitor cell that could be the endocervical reserve cell since these cells express the same panel of keratins [19]. On the other hand, patients can present cervical multifocal lesions with different grade of severities. Genetic alteration analysis of these lesions highlighted allele-specific losses suggesting that distant lesions are in fact related and probably share a common precursor cell [20]. With the discovery of the role of oncogenic HPV in cervical cancer and the paradigm that HPVs infect basal keratinocytes trough epithelium disruption [21], the following question emerged: why SCC are more frequent than vaginal cancers. Recently, a small cell population located at the squamocolumnar junction (figure 1) was identified and potentially represents the first cells that are infected by HPV giving rise to intraepithelial lesions and cervix cancer (figure 2) [22]. These cells display particular cuboidal morphology and form a single layer of epithelial cells joining the endocervical glandular epithelium to the TZ. Interestingly, squamocolumnar junction-specific biomarkers (cytokeratin7, AGR2, CD63, MMP7 and GDA) are expressed in almost all high-grade cervical intraepithelial lesions, SCC and AC, suggesting a common cellular origin for all uterine cervical cancers [22] and a particular susceptibility of these cells to oncogenic potential of HPV proteins.

**Early proteins E5, E6 and E7 exhibit a variety of oncogenic properties**

HPV consists of a double-stranded circular DNA genome coding for early and late genes. Early oncoproteins E5, E6 and E7, are responsible for the occurrence of cervical intraepithelial lesions, whereas E1 and E2 early proteins are implicated in the initiation and regulation of the virus cycle, including repression of E6 and E7 proteins [23]. Late genes encode for the two structural proteins, L1 and L2, forming the viral capsid. HPV virions infect basal epithelial cells of the cervix and need the epithelium differentiation process to produce their late proteins in the upper layer of the epithelium. Then, if infection persists, the viral genome can integrate into the host genome inducing overexpression of oncoproteins, by deletion of E1 and E2, and cancerous transformation of infected cells (reviewed in [24]). As already mentioned, one of the classifications of HPVs resides in their capacity to induce or not neoplasia [25]. Although E6 and E7 from low-risk HPVs interact with their respective targets, they induce lower degradation of these compared to E6 and E7 from high-risk HPVs [26,27]. The major transforming activity of oncogenic HPVs resides in the E6 and E7 genes whereas E5 displays weaker transforming properties.
Although the oncoprotein E6 has no enzymatic activities it induces malignancy by interfering with many cellular proteins. The main oncogenic effect of E6 is provided by the binding to an ubiquitin ligase, E6-AP, leading to the degradation of p53, the major protein implicated in the control of the cell growth arrest and apoptosis after DNA damage [28,29]. Moreover, this viral protein actively contributes to keratinocyte immortalization by inducing overexpression of hTERT [30]. Since the amino acid sequence of E6 contains a PDZ binding motif, many cellular proteins containing PDZ domains were identified as potential targets of E6 [31]. The first identified substrates containing a PDZ domain were the human Disc Large (Dlg) and Scribble (Scrib) proteins, two tumor suppressors involved in the cell polarity control [32,33]. Later, studies have shown that degradation of Scrib proceeds via E6-AP-dependent ubiquitination [33], whereas E6-induced degradation of Dlg has been proposed to involve other ubiquitin ligase than E6-AP [34]. More recently, heparanase overexpression induced by E6 was described in HPV-positive squamous cell carcinomas [35–37]. This enzyme degrades the extracellular matrix and releases growth factors leading to tumor aggressiveness and invasion [38]. Also, an in vitro study demonstrates that the expression of some E6 isoforms (short forms) decreased anti-oxidant activity of the cells inducing an oxidative stress and subsequent DNA damage [39].

Infected cell proliferation is also enhanced through E7 protein transforming activities [40]. By binding the complex pRb/p107/p130, E7 prevents interaction of the complex with the transcription factor E2F resulting in cyclin A and E expressions [41]. More recently, it has also been shown that E2F, by binding to the RRM2 promoter, enhances expression of HIF-1α and VEGF conducting to increased blood vessel density in cervical cancer tissues [42]. In addition, E7 can also abrogate the inhibitory activities of the cyclin-dependent kinase inhibitors p21 and p27.
enhancing expression of E2F-responsive genes and cdk2 activity [43,44]. In response to DNA damages, E7 upregulates Cdt1 and stops cell-cycle at the G2 checkpoint leading to a process of “rereplication” responsible for polyploidy and genomic instability [45].

The main oncogenic function of E5 is the induction of epithelial hyperplasia by sensitizing cells to EGF [46]. It has been shown that E5 can enhance the amount of epidermal growth factor receptor (EGFR) at cell surface by stabilizing it and by reducing its degradation [47,48] leading to stimulation of the EGFR signaling pathway and hyperproliferation of infected epithelial cells [49,50]. The E5 protein also enhances DNA synthesis [47] and increases the motility and invasiveness of a human keratinocyte cell line [51].

The principal and newly identified targets of HPV16 oncoproteins are shown in Table I.

Although HPV oncogenic proteins display numerous transformation activities, their expressions are controlled by E1 and E2 gene products [23] and no cervical cancer cells develop as long as the virus is in the form of an episome in the nucleus of the infected cell. Accidentally, HPV genome can be integrated into the cell genome causing a cut in the E2 gene resulting in the loss of oncoprotein regulation and into cervical cancer cell development. Moreover, E5, E6 and E7 also display immunosuppressive functions (reviewed in [55]) and the persistence of HPV infection, allowing the virus genome integration, is closely depending on the balance between effective immune responses and mechanisms engaged by the virus to escape immune system.

The role of the immune system in HPV infection

Clearance of HPV

The increased incidence and progression of HPV infections in immunodeficient patients illustrate the importance of the cell-mediated immune response in the control of HPV infections [56,57].

The first defense against infections with HPVs and other pathogens is the mechanical barrier of a pluristratified epithelium.
Furthermore, epithelial cells produce a variety of anti-microbial compounds including α-defensins that block nuclear localization of HPV16 [58]. As already mentioned, the majority of the HPV-infected women will clear the viral infection within two years [6]. The innate cells may play an important role in this process including: macrophages, dendritic cells (DCs), natural killer (NK) cells and γδ T cells (reviewed in [59]). Macrophages as well as DCs, are able, at least in vitro, to kill HPV transformed cells [60,61]. More interestingly, NK cells are more numerous in SIL compared to normal exocervix [62]. Like γδ T cells and NK T cells, NK cells are innate lymphocytes able to kill virally infected cells or tumor cells lacking the surface expression of MHC Class I molecules or overexpressing stress-inductible MHC Class I-like molecules (reviewed in [63,64]). NK cells are directly activated by HPV particles. Upon this activation, they secrete high amounts of IFN-γ and become highly cytotoxic against HPV-transformed cells [62]. However, peripheral blood mononuclear cells from patients with active HPV16 neoplastic disease display a reduced NK cell activity against HPV16 infected keratinocytes [65]. The role of NK T cells is still uncertain because the level of circulating NK T cells is not associated with the severity of infection or progression to cervical cancer [66].

As a second line of defense, the adaptive immunity, induced by antigen presenting cells (APC) through a Th1 response, can eliminate HPV-infected cells via cytotoxic T lymphocytes (CTLs) targeting HPV16 E2, E6 and E7 [67–69]. γδ T cells could improve the function of CTLs in the HPV clearance, since they are present in the genital tract and they enhance the Th1 response in the presence of the herpes simplex virus type 2 [70]. In parallel, the specific local humoral immunity of the cervix contributes to the host defense against HPV. In the lamina propria of the endocervix, numerous plasma cells producing IgG and IgA are detected [71]. In animal infection models, cell-mediated immune response is closely followed by seroconversion and production of antibodies against the major capsid protein L1 [72]. In human, seroconversion is detected between 6 and 18 months after the first HPV DNA test in subjects with persistent HPV infection [73]. All these mechanisms of the HPV clearance process are summarized in figure 1.

### Evasion mechanisms of HPV to the immune response

Despite the fact that the majority of the HPV-infected women will clear the viral infection within two years [6], one of the hallmarks of HPV infection persistence is to evade effective recognition by the immune system. The productive life cycle of the virus restricted to the epithelium induces low inflammation since HPVs are not cytolytic. As already mentioned, TZ is the most vulnerable site to tumorigenesis and this vulnerability could be linked to a significant decreased number of Langhans cells (LCs) and altered expression of TNF-α and MIP3α compared to the exocervix [74]. In TZ, the expression of IL-10 and TGF-β is more common than in the exocervix, which suggests that this immunosuppressive condition could be a factor that favorizes progression into cancer [75,76].

HPV proteins downregulate innate immune signaling pathways in the infected keratinocytes [77]. The release of the pro-inflammatory cytokines (especially type I interferons (IFN)-α or -β) is reduced and consequently there is less activation and migration of LCs and less recruitment of stromal DCs and macrophages [78]. Beyond their oncogenic proprieties E6 and E7 proteins induce multiple mechanisms for innate immune evasion. Recently, it is shown that E7 affects the type I IFN response by downregulating activation of TLR9 [79]. E6 protein
also targets the type I IFN pathway by binding to IRF-3 and inhibiting its transcriptional activation function [80]. E6 can also interfere with Tyk2 function, which results in the alteration of the STAT signaling and therefore specifically inhibiting IFN-α-mediated signaling [81,82]. This inappropriate costimulatory microenvironment in SILs seems to induce deficient function of LC [83]. Moreover, in vitro, E6 and E7 can reduce the surface levels of E-cadherin, which can interfere with LC migration [84,85] and LCs are not activated by the internalization of HPV capsids [86]. In contrast, stromal DCs can be activated and stimulate HPV-specific T cells [87].

Also macrophages can be part of the disease progression. Several studies showed that macrophages are present in the uterine cervix and their number is significantly increased in SILs and SCC compared to normal counterparts. These macrophages in the HPV-associated tumors are mainly M2 macrophages. M2 macrophages in tumors are able to disrupt basement membrane allowing tumor growth and metastasis (reviewed in [88]). They also promote the differentiation of naïve T cells to T-regulatory cells through IL-10 [89,90]. APCs of the cervix uptake and process HPV antigens in order to present them to the B and T cells in the lymph node and initiate an adaptive immune response against HPV infection. Adaptive immune response is also targeted by HPV protein, since E5 protein can downregulate the processing of classical MHC molecules to the cell surface, which results in the avoidance of CTL recognition of infected cells [91]. In poorly differentiated adenocarcinomas, the secretion of immunoglobulins is impaired [92].

All these mechanisms of the immunoevasion, summarized in figure 2, can support the establishment of persistent HPV infections, leading to the induction of cervical cancer. Regarding the effects of HPV oncoproteins on the host immune system, it is clear that the immune response needs to be improved to overcome this immunoevasion.

Challenges for immunotherapy of cervical cancer

Currently there are two prophylactic vaccines on the market based on the virus-like particles, which target the low risk types HPV-6 and 11 and the oncogenic types HPV16 and 18 (reviewed in [93]). They are highly efficacious against primary infections due to the high titer of specific neutralizing antibodies that they induce but they have no proven benefits for already infected women and to other oncogenic viruses [94]. To increase the number of HPV targeted by the vaccine and to offer protection against five more HPV types, a nonavalent vaccine is under FDA inspection [95]. To overcome the immunosuppression of the host and to mount an effective immune response that can eliminate HPV-infected and transformed cells, different vaccine strategies have been tested including the use of peptides, proteins, DNA, viral or bacterial vectors and cell-based vaccines that can be combined (reviewed in [96]). Also the synthetic long peptide (SLP) vaccine showed an excellent treatment profile in animals [97]. In patients with advanced or recurrent HPV16-induced gynecological carcinoma, the HPV16-SLP vaccine was well tolerated and induced a broad IFN-γ-associated T cell response but induced no tumor regression nor prevented for a progressive disease [98].

Adjuvant immunotherapies can also improve the current treatment. These therapies involve the administration of cytokines, such as GM-CSF or IFN-β. For example, in a phase I clinical trial, application of GM-CSF on the cervix results in a significant increase of antigen presenting cells and infiltration of cytotoxic T-lymphocytes without recruitment of regulatory T cells [99].

Treatments with IFN-β provide also several new insights. IFN-β induces microRNA-129-5p which downregulates HPV-18 E6 and E7 by targeting SP1 in the cervical cancer cells [100]. IFN-β upregulates A3s, which induces hypermutation of HPV16 E2 in the cervical keratinocytes [101]. Finally, IFN-β also seems to induce senescence by affecting the p53 transactivating activity [102].

As already mentioned, CTLs are necessary to eliminate HPV-infected cells [67–69]. These cells need the help of specific CD4+ T cells. Thus, Scholten et al. [103] tested the feasibility to generate HPV16 specific CD4+ T cells using either transfer of either a MHC class I or MHC class II restricted TCR directed against HPV16 antigens. An alternative strategy could be intravaginal immunization with vectors containing HPV epitopes to induce tissue-resident CD8+ T cell responses [104]. Antibody therapy could be also interesting. For example, in animal models, subcutaneous injection of intrabody against E7 of HPV16 blocks tumor growth [105].

All these strategies can be applied to treat patients suffering from cervical cancer and other HPV16 induced malignancies.

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Origin and immunoeescape of uterine cervical cancer


