Critical role of E6 oncoprotein in cervical cancer: involved in cell proliferation, angiogenesis and apoptosis
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Abstract
Cervical cancer represents one of the most common malignancies in women worldwide and predominantly affects women of poor socioeconomic status. Persistent infection with high-risk human papilloma virus (HPV) is an essential factor for development of cervical cancer. The advent of functional genomics and proteomics has provided hope of discovering novel biological markers for use in the screening, early diagnosis, prognostication and prediction of response to therapy. E6 and E7 oncoproteins of HPV have been known to play major roles in malignant transformation of cervical cells. Accumulating evidences have suggested that E6 oncoprotein may also contribute to cervical carcinogenesis through modulating cellular signaling pathways. Multiple mechanisms, including activation of EGFR or inflammatory cell signaling pathway, have been implicated in malignant transformation by HPV. Therefore, targeting E6 may be a rational approach for chemoprevention and treatment of cervical cancer, and understanding its oncogenic processes may help us to design novel therapeutic strategies. In this review, we discussed the roles of E6 oncoprotein in cervical carcinogenesis, altering several cellular signaling pathways involved in cell proliferation, angiogenesis and apoptosis.

Keywords: HPV; E6; Cervical cancer; Oncoprotein

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Introduction

Worldwide, nearly 530,000 new cases of cervical cancer are diagnosed annually, and 274,000 women die of the disease each year [1]. Cervical cancer is the second most common cancer in women worldwide, and it is the principal cancer of women in most developing countries, where 80 percent of cases occur [2, 3]. Among the 15 oncogenic types identified, HPV-16 and HPV-18 are the most common and cumulatively account for approximately 70% of invasive cervical cancer cases [4].

According to the most recent reports, in the United States of America (USA) women are prone to this infection twice than men in all regions of the world including USA and the prevalence of HPV is much higher in women than men. The reported overall prevalence rate of HPV in women irrespective of races was 17.9%, while men showed a comparably lower rate of 8%. African Americans had the ratio of 20 to 29% as Caucasians and their prevalence rate was known to be about 12.5%. People who had multiple sex partners during their lifetime were on a higher risk of HPV infection as the frequency of HPV was seen to be 20.1% compared 7% in those patients who had only one sexual partner throughout their sexual lives [5]. HPV integration into the host genome is a critical step in cervical carcinogenesis and is found in almost all invasive cervical cancers. Several meta-analyses on HPV presenting an overall HPV prevalence by geographical area ranging from 79.3% in Asia to 88.1% in North America [6], the HPV positivity increased to 87% (ranging from 86% to 94% by region). These variations or the increase of the HPV positivity could be explained by the differences on the methodologies used to determine HPV DNA positivity, histopathologic quality of the samples and the type of specimens analyzed (biopsies, surgical specimens, fresh/frozen tissue samples) [7, 8].

Integration frequently disrupts HPV E2 (E2 regulatory protein) gene expression, leading to increases in E6 and E7 viral oncoproteins, which in turn promote cellular immortalization and transformation. E6 and E7 (E7 transforming protein) gene overexpression contribute to marked genomic instability, accumulation of secondary mutations, and malignant transformation [8, 9]. In addition, the virus integrates into host genes and regulatory elements, which may cause structural alteration of the host genome and transcriptional deregulation of gene expression [10, 11]. The sites of
integration occur frequently at common fragile sites in the genome, but these sites may be less random than originally appreciated. Further evaluation of HPV integration sites by the various HPV types could shed light on additional cancer-causing genes [12, 13].

In this review, the interaction of E6 oncoprotein with other protein direct related in cell proliferation, angiogenesis and apoptosis of cervical cancer.

Oncogenic potential of Human Papillomavirus

HPV 16 affects approximately 20% of the adult population particularly in western countries [14]. It sometimes causes warts which are flat in appearance called condyloma planum and they appear to be less visible. Others include cauliflower like warts mostly seen in the genital areas [15, 16]. The three major onco-proteins which have gained importance in HPV and its relation with cervical cancer are E5, E6, E7 respectively. These proteins are associated with cervical cancer studied both in vitro and in vivo [17]. The proteins E6 and E7 are important for the inhibition of tumour suppressor genes namely p53 and pRb [18, 19].

As far as E5 is concerned it is known to have weak oncogenic properties which result in increased activity of epidermal growth factor receptor (EGFR) and there by inhibiting the major histocompatibility complex expression [20, 21]. For a crucial step to initiate in carcinogenesis it is very necessary that the viral DNA integrates into the host genome. To understand this concept of integration leading to malignancy it is very important to jot down that E2 and E1 are the two proteins which play a leading role in control of transcription and replication and in the absence or loss of activity of these proteins deregulation of E6 and E7 onco-proteins results [22, 23].

E6 Protein

E6 protein is consistently expressed in HPV-carrying anogenital malignant tumors [24] and able to immortalize a wide variety of human cell types cooperatively [25]. One known function of E6 is that it is necessary for viral episomal maintenance, as mutants lacking a functional E6 protein are unable to persist within the cell. The precise activity of E6 that is necessary for this function is not yet clear. E6 is a 151 amino-acid peptide that incorporates a type 1 motif with a consensus sequence -(T/S)-(X)-(V/I)-COOH [26].

E6 is of particular interest because it appears to have multiple roles in the cell and to interact with many other proteins. Its major role, however, is to mediate the degradation
of p53, a major tumor suppressor protein, reducing the cell's ability to respond to DNA damage [27].

HPV16 E6 have been constructed and characterized in a range of studies from different laboratories. A number of interactions have been reported between high-risk E6 and host-cell proteins that provide some clues for the understanding of viral oncoprotein functions. White EA, et al studied the HPV16 E6 changes by conducted an unbiased mass spectrometry analysis for the HPV E6s from different types [28]. Due to the availability of viral vectors when the 16E6 mutant retroviruses were constructed, the E6s used were tagged at their N terminus with both Flag and HA epitope tags. The wild type and each of the three mutant HPV16 E6s were stably introduced into N/Tert-1 cells and processed by immunoprecipitation-MS/MS and CompPASS analysis (Fig. 1).

**USP15 and HERC2 interact with E6**

Vos RM, et al generated a cell line suitable for TAP of protein complexes containing HPV16 E6 by established cell lines using SiHa, an HPV16-positive cervical cancer cell line [29]. They demonstrated that the FLAG-HA-tagged E6 was functional for E6AP binding and p53 degradation in C33A cells. They identified several novel E6-interacting proteins and their results show that the DUB USP15 interacts with HPV16 E6, and this interaction was confirmed following tandem IP of tagged E6, followed by Western blotting against endogenous USP15 in the tagged E6-expressing SiHa cells. Further, identified the 528-kDa protein HERC2, which contains a HECT-like domain and is a putative E3 (Fig. 2).

The differences between the E6 and E7 proteins of high- and low-risk HPV types seem often of a more quantitative rather than a qualitative nature [30]. E6 acts as repressor of apoptosis and mediates survival of severely damaged cells, while E7 functions as promoter for replication and cell growth. Both can independently immortalize human cells, but their joint function gives rise to an interesting complementary and synergistic effect, inducing a marked increase in transforming activity (Fig. 3).

**HPV E6 oncoprotein inhibition and a potential therapeutic target**

There are two prophylactic vaccines are designed to induce the generation of antibodies against the L1 and L2 capsid proteins of HPV in the hopes that these antibodies will
neutralize future virus infection. These vaccines, if administered before exposure to HPV, are reported to be highly effective at protecting women from infection [31].

Successful infection with HR-HPV is usually accompanied by persistent expression of the E6 oncoprotein [32]. The vaccines that have been generated include those that are viral/bacterial based, peptide based, protein based [33].

Therefore, a more in-depth understanding of the molecular interactions of E6 that lead to oncogenesis is being pursued, which will allow for the development of additional approaches to either inhibit E6 expression or combat the consequences that arise from E6 activity. For example, the literature describes experiments to block expression of E6 in HPV-positive cancer cells by inhibiting viral transcription [34], by administering anti-sense constructs [35], and by administering RNAi targeting E6 in cell culture.

The results obtained indicate that these approaches can lead to an absence of detectable transcript, an increase in the tumor suppressor p53 and a concomitant increase in the induction of apoptosis, characteristics of a more normal phenotype. Co-immunoprecipitation assays demonstrate that antibodies generated to recognize epitopes in the N-terminus of E6 blocked E6 association with p53. Similarly, antibodies generated to recognize the second zinc finger domain on E6, which is required for E6-AP binding, blocked E6/E6-AP association and prevented E6-mediated p53 degradation [36]. Monoclonal antibodies to the N-terminal ten amino acids of E6 were shown to specifically bind to the oncoprotein and inhibit proteolysis of p53 in HPV-16-positive CaSki cells, as shown by immunoblot [37].

Peptide aptamers, molecules designed to selectively bind to a given target protein and block its activity in vivo, have been effectively used to inhibit E6 binding to its respective partners, resulting in increased p53 levels and the apoptotic elimination of HPV-positive SiHa cells [38].
Figure 1.
HPV16 E6 mutant proteins exhibit distinct binding profiles. (A) Heat map representing protein-protein interactions identified by immunoprecipitation-MS/MS and CompPASS analysis when the wild type (wt) or one of several mutant forms of FlagHA-HPV16 E6 was used as a bait. Colors in the heat map represent NWD-scores, where an NWD-score ≥ 1 defines a high-confidence interaction. Cells were treated for 4 h with 30 μM MG132 (+) or DMSO control (−) prior to harvest. HCIPs were arranged using a Manhattan distance hierarchical clustering analysis. (B) N/Tert-1 cells expressing the wild type or mutant HA-HPV16 E6, FlagHA-HPV16 E6, or HPV16 E7-FlagHA were treated for 4 h with 30 μM MG132 (+) or DMSO control (−), harvested, and subjected to immunoprecipitation with HA antibody. Immunoprecipitates were separated by SDS-PAGE and Western blotted using antibodies to HA, E6AP, p53, Scribble, or actin. Top panels, whole-cell lysates. Bottom panels, anti-HA immunoprecipitate [28].

Figure 2.
USP15 and HERC2 interact with HPV16 E6. (A) SiHa cells stably transfected with either parental plasmid pOZN or the dual-tagged E6 expression plasmid pOZN16E6 were lysed under mild conditions, and complexes containing E6 were tandem affinity purified. Mass spectrometry analysis revealed specific interaction of E6 with E6AP, USP15, and HERC2. The asterisk indicates the region of the gel analyzed by targeted ion mass spectrometry for the identification of HPV16 E6. Molecular weight (MW) standards are listed to the left of each gel. (B) The dual-tagged HPV16 E6 was tandem immunoprecipitated, followed by Western blotting to confirm the E6-USP15 interaction. (C) The dual-tagged HPV16 E6 was tandem immunoprecipitated, followed by Western blotting to confirm the E6-HERC2 interaction [29].
Figure 3.

Cellular interactions of E6 and E7 oncoproteins and their synergy in induction of cell immortalization. E6 activates telomerase and SRC kinases, and inhibits p53 and BAK. E7 inhibits pRb, with consequent release of E2F and upregulation of p16, which is inactivated by E7. In addition, E7 stimulates cyclins A and E, inactivates CKIs p21 and p27 and induces centriole amplification. E6 and E7 synergize in cell immortalization (dotted lines); E6 prevents apoptosis induced by high E2F levels, while E7 shields E6 from inhibition by p16 [30].

Conclusions

Cervical cancer is a worldwide public health problem among women. To improve the control of cervical cancer, new adjuvant diagnostic and therapeutic strategies are required. Advances in immunology, genomics and proteomics have accelerated our understanding of the genetic and cellular basis of many cancer types. Cervical cancer is a member of the virus-related neoplasms, with its initiation and promotion associated with persistent infection of oncogenic HPV. The integration of molecular and proteomic biotechnology with immunology has also yielded promising findings that may translate into clinically relevant biological assays.

The studies of host proteins associated with HPV E6 oncoproteins continue to display potential future usage in the management of cervical cancer. Subsequent scientific investigations will probably yield new diagnostic and prognostic tools for cervical cancer, provide insights into its underlying biology and contribute to the development of novel management strategies.

Competing interests

The authors declare that there is no conflict of interest.
Authors’ contributions

All authors have been involved in revising critically the manuscript and has given final approval of the version to be published.

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