

Ariel Green

The Role of High Risk HPV E6 Protein in Cervical Cancer Formation

»

Background and natural history:

Over ninety percent of cervical cancer across the world is caused by a virus (2). This notorious culprit that ranked second in cancer-related deaths among females across the world is named human papillomavirus (HPV) (2). HPV is a sexually transmitted, lysogenic, double stranded circular DNA (dsDNA) virus with approximately eight thousand base pairs (1, 9, 15, 16). HPV does not possess an envelope and has icosahedral capsids (18). It, like other papillomaviruses in its taxonomic family, both assembles and replicates in the nucleus of the host cell (17).

The basal cells of the stratified squamous epithelium and the metaplastic cells at the squamocolumnar junction in the cervix serve as HPV's targets (15). Dissimilar to other types of genitourinary infections, HPV does not induce immediate symptoms upon infection (15 – 16). This is due to the host immune response to HPV infection (15). Given the recent discovery of a vaccine against this pathogen, various groups outside of the scientific community have turned their focus onto this virus. However, I would like to focus on the molecular infiltration this virus inflicts upon cervical epithelial cells.

HPV has a genome with three regions, the early (E), late (L), and non-coding long control region (LCR) (1, 9). The late region encodes for the structural proteins of the virus, while the LCR region, as implicated in the name, is for open reading frame (ORF) expression control (1).

It must be noted that not every strain of HPV is carcinogenic

(1). There are over one hundred types of the HPV virus (16). Current epidemiological studies have determined that the following are low-risk types: 6, 11, 40, 42, 54, and 57 (16). These types limit their effects to genital warts that are benign lesions (16).

The most commonly implicated carcinogenic, high-risk, HPV types are the following: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, and 58 (3, 16). These types induce dysplastic lesions, some of which are malignant cancers (16). HPV-16 is found in over fifty percent of cervical cancers (15). Integration of the viral genome into the host cell is required for transformation into cancerous cells (5). This may be due to the loss of E2 and E1 following integration, an ORF product responsible for repressing both E6 and E7 (1). In epithelial cells infected with low risk HPV, the virus remains in the episomal form with the E2 and E1 ORF products intact (15). Without these repressors, the uncontrolled expression of E6 and E7 leads to the degradation of p53 and retinoblastoma (16).

The early region is responsible for the pathogenicity of the virus. Amongst its six genes, two, E6 and E7, are very effective oncogenes. This paper will primarily focus on the role of E6 protein (pE6) in transforming the epithelial cells of the cervix into malignant carcinomas.

The E6 transcript was found in over ninety-two percent squamous cell carcinoma samples taken from Norwegian women (3). It is approximately 150 amino acids long with two zinc binding domains with the CXXC motif (18). The E6 ORF is expressed soon after infection with the HPV virus (4). The HPV virus uses this ORF product to disable the host cell's sensory mechanisms for DNA damage (4). By disabling important checkpoints in the cell, disrupting communication, and other harmful events, this double stranded DNA virus is able to transform dormant epithelial cells into proliferating, immortal carcinomas (4).

High Risk HPV pE6 targets p53 for destruction by ubiquitination:

While pE6 has several mechanisms it uses to accomplish the overall goal of cellular transformation, its most famous pathway is the degradation of the genome protector, p53. This action was shown by

Demers et al (4). They demonstrated that transfection of HPV-16 pE6 into primary human keratinocytes induced the elimination of p53 (4). The removal of p53 in the cell allows for the accumulation of mutations that contribute to the genomic instability of the cell and lead to transformation (11).

P53 is known to play an important role as a checkpoint in the cell cycle (4). It functions in the cell as a transcription factor for other genes that regulate the cell cycle (6). It acts as the signaling molecule to allow progression into both the synthesis phase and mitosis (4). This allows the cell to assess whether or not to replicate its DNA. If the DNA has, by some means, been damaged, p53 signals for cell cycle arrest and apoptosis. It does this by up-regulating the expression of p21 (18).

After treatment with low doses of actinomycin D, which induces DNA damage, epithelial cells that had been infected with this oncogene were unable to halt the cell cycle and continued into the synthesis phase (4). This function plays a significant role in the carcinogenicity of this ORF product. HPV-16 pE6 binds with a host E3 ubiquitin ligase given the name of E6AP (5, 7). They, cooperatively, bind to p53 and signal for degradation by the proteasome complex (5). To accomplish this task, HPV-16 pE6 requires the presence of six amino acids, out of the entire 150 amino acid sequence, that contain a PDZ binding domain (5).

After transcription of the E6 gene, it undergoes splicing (11). One of the splicing patterns gives rise to E6*I, which can bind to full length pE6 (11). This binding controls the degradation of p53 during the course of the disease to prevent transformation into cancerous cells (11). Hence, mRNA processing can affect the carcinogenicity of HPV. In this way, HPV can regulate the expression of pE6 and determine when to transform the cells by mechanisms other than E2 expression (11). However, despite these posttranscriptional modifications, all of the infected cells were transformed into carcinomas over time (11).

Therefore, by eliminating the cell's ability to assess DNA damage, epithelial cells continue through the cell cycle. This alone is able to transform normal epithelial cells into cancer. Unfortunately, the high risk types of HPV have other mechanisms they use to further

the transformation into cancerous cells (4). In combination with this carcinogenic blow, pE6 begins the process of transformation strong.

High Risk HPV pE6/E6AP degrade PDZ domain containing proteins involved in tumor suppression:

Proteins containing the PDZ domain have been implicated in tumor suppression (10). In epithelial cells, PDZ proteins are generally located at areas of cell-to-cell contact, such as tight junctions (18). Their role is thought to aide in signal transduction between cells (18).

DLG4 is a known target of HPV-18 pE6 and HPV-16 pE6 (10). Similar to the degradation of p53 by the proteasome complex, pE6 targets DLG4, perhaps in conjunction with E6AP, for degradation by process of ubiquitination (10). In CaSki cells, DLG4 is known to have tumor suppression capabilities, and thus its degradation in HPV infected cells may be important in transformation (10). The complete mechanism by which DLG4 functions as a tumor suppressor is still unclear (10). Still, it is, obviously, important to note that pE6 can disable another tumor suppressor besides the p53.

High Risk HPV pE6 binds to coactivators CBP /p300 to prevent p53 acetylation:

Interferon is a signaling molecule the host cell produces in response to viral infection (6). Type I interferons not only signal for an immune response, but can also induce growth arrest (6). Interferon is linked with p53 in that p53 can influence the production of interferon by up-regulation and therefore, the host cell's response to viral infection (6).

The activity of p53 is determined by coactivators CBP/p300 (6). They function as acetyltransferases for histones (6). Histone acetylation is very important for the expression of genes. By weakening the electrostatic interaction between DNA base pairs and the lysine residues on histone tails, the DNA encoding genes becomes accessible (7). These coactivators do not only acetylate histones, they also acetylate p53 itself (6). Acetylation of p53 increases its stability and transcriptional activity (6).

Not only does pE6 aide in the ubiquitination of p53, but it also diminishes p53's transcriptional activity as well by binding to CBP/

p300 (6). This sequestering activity prevents p53 acetylation (6). This decreased stability of the p53 weakens the remaining, non-ubiquitinated p53's ability to halt the cell cycle. This is yet another mechanism by which high risk HPV virus eliminates cellular defense against uncontrolled proliferation.

High Risk HPV E6 indirectly disrupts interferon response by infected cells:

Among the various regulatory effects the p53 gene has, interferon response is one of them. When infection occurs, HPV E7 protein (pE7) stimulates the formation of interferon (6). This is, of course, detrimental to the virus. HPV, unfortunately, has a counteractive mechanism. PE6 eliminates the antiproliferative response the infected cell would have by eliminating the effects of the p53 through binding to CBP and p300 (6).

P53 regulates the type I interferon response (6). As mentioned before, type I interferons can induce an arrest in cell growth (6). Non-acetylated p53 is unable to mediate the sensitivity of the infected cell to these interferons (6). PE6 induces this state of p53 by forming a trimeric complex with CBP, p300, and p53 (6). Therefore, infection by HPV bypasses antiviral systems that are functioning correctly.

Although the cells are properly responding to the HPV infection, they are no longer sensitive to the signal (6). This could create problems in treating HPV infections with interferons. If pE6 and pE7 are expressed without control, as they are after integration, then interferon treatment may not be effective. However, after early infection, interferon treatment could, potentially, be a reliable means of eliminating HPV infected cells.

High Risk HPV pE6 induces polyploidy:

A common form, in solid tumors, of genomic instability is aneuploidy, which is thought to form from a tetraploid intermediate (8, 14). Aneuploidy is a condition where the cell does not have a multiple of the haploid chromosome number. This condition is thought to be responsible for the different phenotypes of cancerous cells including the following: abnormal size, loss of contact with neighboring tissues, resistance to chemotherapy, increased proteins on the outer membrane, morphology, and others (14). Aneuploidy forces the cell to enter into

a never ending cycle of redistributing genes to gain the most beneficial translational products, even at the expense of genomic stability (14).

Tetraploidy is thought to arise when, during mitosis, cells pause in the metaphase point for extended time periods (8). This is independent of a spindle checkpoint (8). The function of the postmitotic checkpoint is similar to the G1 and G2 checkpoints. In every instance, p21 is present and is mediated by p53 expression (8). Normally, these abnormal cells would not be able to continue through the cell cycle. However, given the absence of p53 and pRb in HPV-induced carcinomas, the cells progress to the synthesis phase of the cell cycle (8).

pE6 is known to cause polyploidy by preventing cell cycle arrest stimulated by microtubule disruption (8). This contributes to the genomic instability of HPV infected cells, which leads to the development of carcinomas (8). The abrogation of p53 would seem a likely pathway to mediate this activity, but studies in defective p53 cells demonstrated that pE6 still induced polyploidy (8). This is important because degradation of pE6 is never complete in cervical cancer cells (8). Therefore, pE6 must have some other means of inducing polyploidy in the presence of the p53. Hence, even without the degradation of p53, pE6 is able to induce genomic instability that leads to carcinomas in human epithelial cells. In conjunction with the p53 degradation in human epithelial cells, pE6 further contributes to the transformation of these cells into cancer.

High Risk HPV-16 E6, in conjunction with HPV-16 E7, demolishes the expression of TLR9:

Toll-like receptors (TLRs) are responsible for recognizing molecular patterns associated with pathogens on both non-immune and immune cells (9). They are the receptors that bind to domains that originated from pathogens (9). TLR9 is responsible for recognizing foreign dsDNA-derived CpG motifs (9). Given the fact that HPV is a dsDNA virus, tests were done to ascertain whether TLR9 could bind to DNA motifs arising from HPV. The results established that TLR9 can recognize DNA motifs from HPV (9).

In HPV-16 infected cells, pE6 and pE7 were able to down-regulate the TLR9 pathway (9). This is not done by process of

ubiquitination or protein alteration (9). Instead they eliminate yet another sensory mechanism by halting transcription of TLR9 by down-regulating its promoter (9). Hence, pE6 not only bypasses the sensory mechanisms of the cell, but it evades the defense mechanism of the body by removing the receptor that could have detected its presence. This prevents any interference from the immune system that would quickly dispose of the virus before its most damaging work could be done (9).

High Risk HPV-16 E6 activates hTERT:

Telomerase, a four-subunit enzyme, is known to be involved in the immortalization of cancer cells (12, 18). It acts as the catalytic subunit of telomerase (12). By extending the telomeres, telomerase reverse transcriptase (hTERT) enables cells to bypass the Hayflick limit and prevent cell senescence (12 - 14). Cell senescence occurs when, after approximately fifty replications, the telomeres are shortened to the extent that chromosomes begin to fuse with each other and become aneuploid cells (13, 14). This triggers cell apoptosis if the damage cannot be fixed (13). Telomerase uses RNA as a template, synthesizing hexamer repeat DNA to the 3' end of telomeres (13, 18). This effectively lengthens the telomeres, prevents chromosomal fusion, and halts the signaling of apoptosis (13, 18). In most somatic cells, hTERT is down-regulated to prevent immortalization (13). In over ninety percent of cancerous cells, however, hTERT is active (13). Telomerase may also have proliferative activity independent of telomere extension as well (12).

HPV pE6 activates the TERT gene in cervical epithelial cells (12). In normal epithelial cells, histone deacetylases (HDAs) inactivate the hTERT promoter (12). By inhibiting HDA activity, the hTERT promoter can be activated (12). PE6 activates the hTERT promoter when H3 is acetylated; this is further enhanced by down-regulation of p300 and is dependent upon E6AP (12). This process begins early on during HPV infection, and continually increases until the increased transcription of hTERT is no longer dependent upon E6 (12). This trend follows the relative immortalization of the epithelial cells (12).

The activation by pE6 of hTERT is both p53-degradation and PDZ domain binding independent (12). This function is dependent

upon E6AP ligase (12). Although p300 knockdown is not the sole reason for hTERT up-regulation, it does seem to play an important role in telomerase activation (12).

In summary, pE6 has the capability of activating cellular machinery that immortalizes the cell. This transforms epithelial cells that previously had a timed death into cells that proliferate without consequences. With the absence of p53 and pRb, DNA damage accumulates and telomerase prevents shortened chromosome ends from fusing with neighboring chromosomes. Therefore, it is yet another mechanism by which pE6 bypasses cellular checkpoints for DNA damage and, instead, transforms into carcinogenic cells.

Discussion:

Given the examples of how a single protein infiltrates several elements of the cellular defense machinery, it is no wonder that a system of these proteins induces transformation of normal cervical epithelial cells into cancer. HPV is a virus that contains all of the necessary elements to hijack the cell into virus-making factories. HIV disrupts the immune system. HPV disrupts the cell.

Stripping the cells of their original function, HPV quickly disposes of the genome protectors. It disrupts both intracellular and intercellular communication. It activates genes for immortalization that, in a way, return the cells to an undifferentiated state. It induces genomic instability. All of these things are done within the first stages of infection.

Every year, approximately 6.5 million new HPV infections are reported in the United States alone (15). An estimated 20 million individuals are already infected with the virus (15). It is estimated that at least fifty percent of all sexually active individuals will acquire the virus by the age of 50 (15). To think that cervical cancer is caused by a virus of this competency is not a welcome thought. Causes for cancer have always been elusive or too ambiguous, but to find a culprit, and a culprit that is highly efficient at transforming these cells, has spurred research around the world. Recently, a vaccine has been introduced that may hold the key for prevention, but treatments for this deadly virus still fall in the category of barbaric killing of cells, without much differentiation between

the infected and the normal.

HPV remains a virus of interest, not only for clinical reasons, but for the understanding of the cell cycle in humans. HPV abolishes checkpoints in the cell cycle through various mechanisms. Further understanding of the viral mechanisms of transformation could enlighten researchers about the pathways cells take to become cancer without viral influence. This information could be vital in developing new cancer treatments and more insight into cell senescence in general.

If scientists can understand the viral pathway high-risk HPV uses to transform cervical epithelial cells into carcinomas, then they can understand more about cellular immortalization. HPV-infected cells provide an excellent source for testing telomerase activity, not only for cancer treatment, but for potential uses in prolonging life.

Works Cited

- #1 Tungteakkhun, S.S., and Duerksen-Hughes, P.J. Cellular binding partners of the human papillomavirus E6 protein. *Archives of Virology*: 397 – 408, 2008.
- #2 Wei, Qize. Ptx2a binds to human papillomavirus type 18 E6 protein and inhibits E6-mediated p53 degradation in HeLa cells. *Journal of Biological Chemistry*. 37790 – 37797, 2005.
- #3 Kraus, I, Molden, T, Holm, R, Lie, A.K., Karlsen, F., Kristensen, G.B., and Skomedal, H. Presence of E6 and E7 mRNA from human papillomavirus types 16, 18, 31, 33, and 45 in the majority of cervical carcinomas. *Journal of Clinical Microbiology*: 1310 – 1317, 2006.
- #4 Demers, W., Foster, S.A., Halbert, C.L., and Galloway, D.A. Growth arrest by induction of p53 in DNA damaged keratinocytes is bypassed by human papillomavirus 16 E7. *Proceedings of the National Academy of Science*.: 4382 – 4386, 1994.
- #5 Cooper, B., Brimer, N., and Vande Pol, S.B. Human papillomavirus E6 regulates the cytoskeleton dynamics of keratinocytes through targeted degradation of p53. *Journal of Virology*: 12675 – 12679, 2007.
- #6 Hebner, C. Beglin, M., and Laimonis, A.L. Human papillomavirus E6 proteins mediate resistance to interferon-induced growth arrest through inhibition of p53 acetylation. *Journal of Virology*: 12740 – 12747, 2007.
- #7 Habold, C., Poehlmann, A., Bajbouj, K., Hartig, R., Korkmaz, K.S., Roessner, A., and Schneider-Stock, R. Trichostatin A causes p53 to switch oxidative-damaged colorectal cancer cells from cell cycle arrest into apoptosis. *Journal of Cellular and Molecular Medicine*: 607-621, 2008
- #8 Liu, Y., Heilman, S.A., Illanes, D., Sluder, G., and Chen, J. p53-independent abrogation of a postmitotic checkpoint contributes to human papillomavirus E6-induced polyploidy. *Cancer Research*: 2603 – 2610, 2007.
- #9 Hasan, U., Bates, E., Takeshita, F., Biliato, A., Accardi, R., Bouvard, V., Mansour, M., Vincent, I., Gissman, L., Iftner, T., Sideri, M., Stubenrauch, F., and Tommasino, M. TLR9 expression and function is abolished by the cervical cancer-associated human papillomavirus type 16. *The Journal of Immunology*: 3186 – 3197, 2007.

- #10 Handa, K., Yugawa, T., Narisawa-Saito, M., Ohno, S., Fujita, M., and Kiyono, T. E6AP-dependent degradation of DLG4/PSD95 by high-risk human papillomavirus type 18 E6 protein. *Journal of Virology*: 1379 – 1389, 2007.
- #11 Cruz-Hernandez, E., Garcia-Carranca, A., Mohar-Betancourt, A., Duenas-Gonzalez, A., Contreras-Paredes, A., Perez-Cardenas, E., Herrera-Goepfert, R., and Lizano-Soberon, M. Differential splicing of E6 within human papillomavirus type 18 variants and functional consequences. *Journal of General Virology*: 2459 – 2468, 2005.
- #12 James, M., Lee, J.H., and Klingelutz, A.J. HPV16-E6 associated hTERT promoter acetylation is E6AP dependent, increased in later passage cells and enhanced by loss of p300. *International Journal of Cancer*: 1878 – 1885, 2006.
- #13 Mazzucchelli, G.D., Gabelica, V., Smargiasso, N., Fleron, M., Ashimwe, W., De Pauw-Gillet, M., Riou, J., De Pauw, E. Proteome alteration induced by hTERT transfection of human fibroblast cells. *Proteome Science*, 2008.
- #14 Rasnick, D. Auto-catalysed progression of aneuploidy explains the Hayflick limit of cultured cells, carcinogen-induced tumours in mice, and the age distribution of human cancer. *Biochemical Journal*: 497 – 506, 2000.
- #15 Ault, K. Epidemiology and natural history of human papillomavirus infections in the female genital tract. *Infectious Diseases in Obstetrics and Gynecology*: 1 – 5, 2006.
- #16 Dehn, D., Torkko, K.C., and Shroyer, K.R. Human papillomavirus testing and molecular markers of cervical dysplasia and carcinoma. *Cancer Cytopathology*: 1 – 14, 2007.
- #17 Zheng, Z., and Baker, C.C. Papillomavirus genome structure, expression, and post-transcriptional regulation. *Front Biosci.*: 2286 – 2302, 2006.
- #18 Longworth, M.S., and Laimins, L.A. Pathogenesis of human papillomaviruses in differentiating epithelia. *Microbiology and Molecular Biology Reviews*: 362 – 372, 2004.