International Journal of Molecular Medicine and Advance Sciences 4 (3): 55-61, 2008
ISSN: 1813-176X

The Mechanisms of HPV-Induced Carcinogenesis and the HPV Vaccine

G. Camilleri and R. Blundell
Department of Physiology and Biochemistry, University of Malta, Msida MSD06, Malta

Abstract: Human Papillomavirus (HPV) is the most common sexually transmitted virus. It is estimated that 75% of sexually active adults transmit HPV at some instance during their life. It has long since been known that infection with particular genotypes of this virus is a necessary factor for the development of cervical cancer. In fact, the DNA of this virus is found in 100% of histologically-confirmed cervical cancers. Cervical cancer is a frequent cause of female morbidity and mortality, especially in developing counties. The first part of this study will focus on the way in which infection with specific genotypes of this virus can lead to the development of neoplasia. This will be done in part by explanation of the life cycle of this virus as well as by clarification of the function of the 2 oncogenes E6 and E7 that this virus possesses as part of its genome. It must be kept in mind that not all genotypes of this virus are carcinogenic. In fact, the genotypes that are most strongly associated with cervical cancer are HPV-16 and HPV-18, 2 genotypes that were described by the International Association of Research on Cancer (IARC) as definite human carcinogens. These two genotypes also fall under the heading of high-risk viruses, also because of their oncogenic potential. Given the fact that infection with HPV is an essential step in the development of cervical cancer, prevention of infection by vaccination can reduce the incidence of this cancer. In June 2006, the Food and Drug Administration (FDA) approved an HPV vaccine, Gardasil, for clinical use in females aged 9-26. This vaccine protects against 4 genotypes of HPV, two of which are the above-mentioned HPV-16 and HPV-18. These 2 genotypes together are responsible for over 70% of cervical cancers. It is thus, hoped that this vaccine will have major benefit on a global scale. The 2nd part of this review will focus on diverse issues related to the HPV vaccine. A brief review of the experiments and trials that were done in the hope of developing an efficacious vaccine is given first, followed by a discussion of the so-called Virus-Like Particles or VLPs. Finally, a review of the two HPV vaccines that were developed, Gardasil and Cervarix, will be given.

Key words: Carcinoma, carcinogenesis, vaccine, VLPs, gardasil, efficacy

INTRODUCTION

A tumour results from the independent and abnormal growth of cells (Underwood, 2004) and occurs when the control mechanisms of proliferation of the cell are disturbed (Bonfiglio and Stoler, 1993). Cancer refers to any one of more than 100 diseases and is the result of a complex mixture of environmental, nutritional and hereditary factors. In fact, carcinogenesis is multistep, multipath and multifocal and is often driven by genetic instability (Lippman and Ki Hong, 2002). About 15% of the ten million cases of cancer that develop annually throughout the world are attributable to infectious agents, with Human Papillomaviruses (HPVs) accounting for approximately 30% of these cancers (Lowy and Schiller, 2006).

The connection between HPV and cervical cancer: A 95-100% of all cervical cancers and a high proportion of other anogenital cancers are caused by infection with HPV (Fig. 1). Over 50% of cervical cancers and high grade CIN lesions contain HPV-16 DNA. On the other hand, HPV-18 causes 10-15% of cervical carcinomas; HPV-45 causes 7% of these lesions whilst HPV-31 causes 3% of cervical cancers. HPV-18 also causes 35% of cervical adenocarcinomas (Khan *et al*., 2005).

Proteins of HPV types 16 and 18 interfere with the functions of cellular regulatory pathways. This fact has been supported experimentally (IARC, 1995). Almost 100 case control studies have been reported that examine the relationship between HPV and cervical neoplasia and almost all have found positive association. This led to the international agency for research on Cancer to draw the following conclusion: HPV types 16 and 18 are carcinogenic to humans; HPV types 31 and 33 are probably carcinogenic to humans; some HPV types other than 16, 18, 31 and 33 are possibly carcinogenic to humans Table 1 (IARC, 1995).

Corresponding Author: Renald Blundell, Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta, Biomedical Science Building, Msida MSD06, Malta

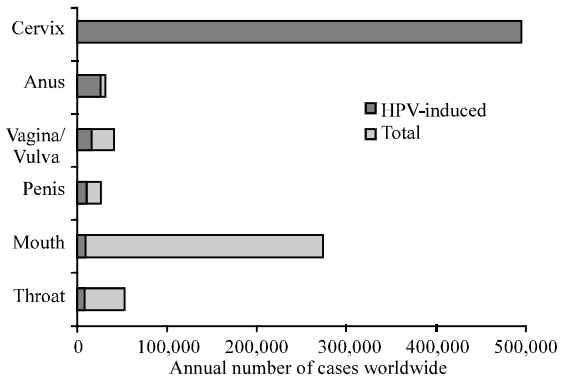

Fig. 1: Bar Graph showing the fact that HPV is involved in a number of different cancers, but it is most strongly associated with cervical cancer. Source: http://en.wikipedia.org/wiki/Image: Cases_of_HPV_cancers_graph. png

Table 1: Gynaecologic lesions associated with human papillomavirus

Type	Lesion
6a-f	Condylomata acuminata; CIN I, II and III; VIN I, II and III
11a, b	Condylomata acuminata; CIN I, II and III
16	Condylomata acuminate; CIN I, II and III; VIN I, II and III; Bowenoid papulosus; Carcinoma of the cervix
18	Carcinoma of the cervix
30	CIN
31	CIN; Carcinoma of the cervix
33	Bowenoid papulosus
	CIN; Carcinoma of the cervix
34	Bowenoid papulosus
35, 39	CIN; Carcinoma of the cervix
40, 42, 43, 44	CIN
45, 51	CIN; Carcinoma of the cervix
52	Carcinoma of the cervix
56	CIN
58	Carcinoma of the cervix

CIN: Cervical Intraepithelial Neoplasia; VIN: Vulvar Intraepithelial Neoplasia; Source: DiSaia and Creasman (2002)

The benefit of a vaccine: The purpose of active immunization is to induce an immune response by giving antigen in such a way that pathogenic effects are avoided but effective immunity is generated. The 1st exposure to antigen, as in vaccination, induces a primary response that generates a primed population of memory cells. Subsequent exposure to antigen leads to a faster and stronger secondary immune response (Onon, 2003). A vaccine is considered to be efficacious if it reduces the incidence of vaccine type-specific persistent infections and of associated cervical intraepithelial neoplasia.

Since cervical tumours are viral in origin, generation of antiviral immunity by vaccination could have major benefits on a global scale. Vaccine development has involved attention on the design of vaccine components (e.g., antigen expression, choice of adjuvants, formulation and patent rights) and the development of serologic tests to measure immunity and tests to detect the specific type of HPV infection. HPV vaccine development has been considerably advanced due in part to the production of Virus-Like Particles (VLPs) (Lowy and Schiller, 2006), which mimic the structure of the HPV virion but do not contain genetic material. They can be manufactured by exogenous expression of L1 in a variety of cell types, including bacterial, yeast, insect and mammalian cells. VLPs are noninfectious and nononcogenic, making them ideal candidates for use in HPV vaccine production.

EXPERIMENTAL EVIDENCE

Experiments supporting, the carcinogenicity of specific HPV types have been performed and the following conclusions were drawn:

- Overexpression of HPV genes in high-risk HPV infected cells leads to chromosomal instability
- Immortalisation of human cells, that is, their continuous *in vitro* growth, is restricted to high-risk viruses. Moreover, chromosomal abnormalities and alterations in ploidy are regularly detected in HPV-immortalised cell lines
- Immortalized cells can subsequently convert to malignant growth. This involves the modification of genes engaged in the control of HPV transcription and which are activated by a paracrine regulatory pathway
- HPV 16 and 18 influence transcription from certain promoters
- The E2, E5, E6 and E7 open-reading frames of several HPV genotypes contribute to the immortalization/ transformation function of the viruses. Transformation is defined as the ability to induce tumours in immunocompromised hosts (IARC, 1995)

THE HPV LIFE CYCLE

HPV infection occurs when the virus enters the epithelium (through a cut or minor injury in the skin) and reaches the basal cells, especially those at the transformation zone. The virus loses its capsid, its DNA enters the basal cell and reaches the nucleus, where it is integrated in the host cell DNA. Therefore, all cells derived from this basal cell will be infected with the virus because they will all contain the viral genome. These infected cells reach the upper part of the epithelium where they start produce the viral proteins and mature viral particles (Palefsky, 2002).

Koilocytosis occurs: Koilocytes are large, rounded vacuolated cells (Craig and Lowe, 2003) with an enlarged and irregular nucleus surrounded by a clear space (Palefsky, 2002). The koilocyte is the histological hallmark of infection with HPV (Craig and Lowe, 2003). During this stage, mild dysplasia-there is rapid viral replication, making this stage the most contagious one. Moderate and severe dysplasias, on the other hand, are characterized by small immature cells because even the top layers of the epithelium, which normally do not replicate, start multiplying. Severe dysplasia is potentially pre-cancerous (Palefsky, 2002).

HPV-INDUCED CERVICAL CARCINOGENESIS

HPV-targeted cellular proteins-p53 and pRB: Alteration in the p53 protein, the product of the tumour suppressor gene p53, is a common event that occurs during the development of most carcinomas. One of the HPV-encoded proteins, E6, can interact with and functionally inactivate the p53 protein. This suggests that somatic alteration within the p53 gene itself may not be a necessary step during the development of HPV-associated cancers. In fact, it has been observed that p53 mutations are extremely rare in HPV-positive cancers. On the other hand, the HPV-encoded oncoprotein E7 has been shown to interact with and inactivate the function of pRB (retinoblastoma protein), a tumour suppressor protein. An inverse correlation between HPV positivity and pRB mutations has also been noted. A high expression level of these viral oncoproteins is needed for the malignant phenotype (IARC, 1995).

Viral DNA integration: Viral DNA can be maintained episomally in the nucleus of the infected cell/s. The detection of this genetic integration in cervical neoplasia is frequently associated with malignant progression. This is because integration is postulated to:

- Deregulate E6 and E7 expression through loss of viral transcription regulators such as E2 and/or escape from cellular control through transcriptional initiation of these regions from flanking cellular promoters
- Increase the stability of the E6/E7 in mRNA, giving cells with integrated viral sequences a selective growth advantage
- Transcriptionally activate cellular proto-oncogenes by viral promoters (IARC, 1995)

Chromosomal alterations: Although, these non-random alterations are present in cytogenetic analyses of cervical carcinomas and although, it is known that they play a role in tumour development, they have been found in both HPV-positive and HPV-negative women. Regions that undergo loss of heterozygosity in cervical cancer include 1p, 1q, 2q, 3q, 4q, 5p, 5q, 6p, 6q, 10q, 11p, 11q, 17p, 17q, 18p (IARC, 1995).

Interaction of HPV with environmental agents: HPV and Herpes Simplex Virus (HSV) may act as syncarcinogens, but this has not been proven. HPV expression in vitro is also influenced by Human Herpesvirus 6 (HHV-6); in fact, HHV-6 increases E6 and E7 mRNAs in infected carcinoma cell lines.

Hormones and antioestrogens alter the expression of the HPV genome. Dexamethasone interferes differentially with the transcription of the E6 and E7 genes in HPV-18-positive carcinoma cell lines. HPV-16 expression is markedly increased in human ectocervical cells exposed to glucocorticoid or progesterone.

The chemotherapeutic agent, tamoxifen, stimulates the proliferation of an HPV-16-positive cervical carcinoma cell line if present at low concentrations. Mutagens and immunosuppressants also cooperate with HPV in various ways. UV and X-ray radiation, by inducing mutations, may increase the expression of viral oncoproteins though direct interference with factors that down-regulate viral transcription or replication (IARC, 1995).

Recent studies and clinical trials

Portland study: This examined data from 20, 514 women, who received routine cytological screening in a prepaid health plan. It was found that women who tested positive for HPV 16 or 18 were diagnosed with CIN III or cervical cancer more often than women who tested positive for another oncogenic HPV type or women who tested negative for HPV. Khan *et al.* (2005) concluded that HPV 16 and 18 are potent carcinogens and should be more effectively targeted in clinical practice.

The ALTS clinical trial: Castle *et al.* (2005) found that in young women with either equivocal or mildly abnormal cervical cytology, having a baseline, prevalent HPV infection was associated with a very high absolute risk of developing Cervical Intraepithelial Neoplasia (CIN) 3 or cervical cancer over a 2 year period. This corresponds to a 5 fold greater risk than the collective risk attributable to other prevalent oncogenic HPV type infections. Castle *et al.* (2005), also demonstrated the potential utility of adjunctive testing of HPV 16 and possibly HPV 18 in a screening population.

THE HPV VACCINE

Studies and trials: Liu *et al.* (2002) developed recombinant Adeno-Associated Virus (rAAV) encoding

HPV-16 E7 Cytotoxic T-Lymphocyte (CTL) peptide DNA fused with heat shock protein (hsp) DNA as a tumor vaccine. This uses hsp as a carrier protein and delivery by rAAV vector.

Emeny *et al.* (2002), demonstrated that a recombinant HPV-11 L1 VLP vaccine could elicit HPV-specific antibody and proliferative T-cell responses in 18-25 year old women not exposed to HPV-11.

Peng *et al.* (2004), investigated the feasibility of the HPV-16 oncoprotein E6 as a target for vaccine development and demonstrated that the linkage of Calreticulin (CRT) to E6 elicits an E6-specific $CD8^+$ T-cell immune response in C57BL/6 mice. Furthermore, vaccination with DNA encoding CRT linked to wild-type E6 can protect mice against challenge with an E6-expressing tumor cell line. This suggests that the amino acid 50-57 sequence of E6 is important for antitumor effects.

According to Baud *et al*. (2004), the development of a Salmonella-based vaccine against HPV infection and associated lesions had the theoretical advantage of inducing long-lasting systemic and mucosal immunity with a single oral vaccination. However, this vaccine was not tested in women due to several drawbacks.

Virus-Like Particles (VLRs): Empty viral capsids, termed Virus-Like Particles (Fig. 2) (VLPs) are synthesized using microbial or cellular expression systems and represent the leading candidate vaccine for the treatment or prevention of cervical cancer in humans. VLPs are purified, concentrated, distributed into aliquots and combined with an adjuvant. Vaccination with L1 VLPs derived from species-specific papillomaviruses neutralizes virus and, in animal models, protects against the development of lesions.

HPV VLPs and chimeric VLPs are immunogens that are able to elicit potent anti-viral/tumor B and T-cell responses. VLPs were found to bind very well to human and mouse immune cells that expressed markers of Antigen-Presenting Cells (APCs) such as MHC class II, CD80 and CD86, including dendritic cells, macrophages and B cells. Dendritic cells, as the most potent inducers of immune responses, play a central role in VLP-induced immunity. It was clearly demonstrated that immature human dendritic cells were fully activated by chimeric HPV16 VLPs and were subsequently capable of inducing endogenously processed, epitope-specific human T-cell responses *in vitro* (Mandic and Vujkov, 2004).

Gardasil and cervarix: Gardasil, produced by Merck, is a quadrivalent vaccine that offers protection against HPV types 6 and 11, which are responsible for 90% of genital warts and HPV types 16 and 18, which are associated with 70% of cervical cancers. According to a phase III study, the vaccine is 100% effective in preventing HPV 16 and 18 related cervical precancerous and cancerous changes. (Mayor, 2005). This is the first vaccine to protect against cancer and against a sexually transmitted disease (Tanne, 2006). The vaccine is formulated with a classic alum adjuvant.

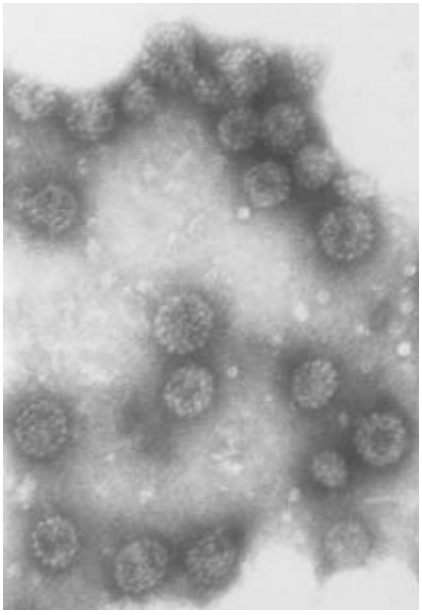

Fig. 2: Human Papillomavirus (HPV) L1 Virus-Like Particle (VLP). Source: http://www.urmc. rochester.edu/ GEBS/faculty/Images/vlps. jpg

Gardasil was approved by the food and drug administration on the 8th of June 2006 and by the European medicines agency in late September 2006 for use in girls and women aged 9-26 years (Tanne, 2006). It has also been approved by regulatory agencies in Australia, Brazil, Canada, Mexico and New Zealand.

Optimal protection against infection with HPV requires a course of three doses of Gardasil, with 2 months between the 1st and 2nd doses and 4 months between the 2nd and 3rd doses. The cost is approximately $120 and €120/dose in the United States and Europe, respectively.

Cervarix, developed by GlaxoSmithKline, is a bivalent vaccine that protects against HPV types 16 and 18. The vaccine is formulated with a new ASO4 adjuvant that contains monophosphoryl lipid A, a derivative of bacterial cell walls. ASO4 is also incorporated in Fendrix (hepatitis B vaccine) and a candidate vaccine against herpes simplex virus, neither of which are approved for use in Canada (Dawar *et al*., 2007).

Efficacy: Prophylactic HPV vaccination is mostly efficacious in women aged 15-25 years, who receive all 3 vaccine doses and who have no prior abnormal results from Pap screening (Rambout *et al*., 2007). For this reason, Gardasil should be offered to females before they are at risk of HPV infection (Dawar *et al*., 2007)

Efficacy against persistent infection is 94 and 80% for Gardasil and Cervarix, respectively. Vaccine efficacy for precancerous lesions caused by HPV types 16 and 18 is 98% for Gardasil and 90% for Cervarix. In addition, Gardasil offers 100% protection against vulvar intraepithelial neoplasia (grade 2-3) and vaginal intraepithelial neoplasia (grade 2-3) caused by HPV types 16 and 18. Gardasil is also 96% efficacious in preventing genital warts. Moreover, in the Cervarix trials, modest cross protection was documented against infection by HPV type 45 (vaccine efficacy 60%) and to a lesser extent, against HPV types 31 and 52 (vaccine efficacy 36 and 32%, respectively).

Both Gardasil and Cervarix are highly immunogenic. In fact, the vaccine-induced antibody titres are much higher than those induced by natural HPV infections. Gardasil-induced antibody titres peak 7 months following the start of the vaccine series. The titres then decline and reach a plateau 18-24 months later. This plateau is maintained for at least 5 years, with 5 year levels that are similar to the titres naturally induced by HPV types 6 and 18 and that are higher than the titres naturally induced by HPV types 11 and 16. Cervarix-induced antibody titres follow the same profile as Gardasil, but the 18 month plateau level is many-fold higher than the levels induced by natural infection and after 51-53 months, 100% of women remain seropositive for both HPV types 16 and 18.

Titres for both vaccines are 1.7-2.4 times higher in young adolescents than in women aged 16-26 years. In fact, 2 doses of Gardasil (0 and 2 months) in adolescents aged 10-15 years produced antibody titres that were equivalent to those produced by 3 doses (0, 2 and 6 months) in women aged 16-26 years for 3 of the 4 vaccine genotypes (Dawar *et al*., 2007).

Some scientists consider the prevention of HPV infection in men (by vaccinating them), not only to reduce rates of anal cancer and genital warts in men, but also to reduce cervical cancer in women. Studies are being conducted as regards this issue (Geipert, 2005).

Drawbacks: The vaccine might be too expensive for developing countries, which have much higher death rates from cervical cancer than do developed countries, largely because they lack organized screening programs. Also, although, Gardasil protects against infection with HPV6, HPV11, HPV16 and HPV18, it will not prevent individuals already infected with either HPV16 or HPV18 from developing cervical cancer, which can occur up to 10 years after infection. It will also not prevent individuals infected with other oncogenic strains of HPV from developing cancer (Honey, 2006). For this reason, all vaccinated females should continue to participate in Pap smear screening programs because they remain at risk of adverse gynecological outcomes from other high risk HPV genotypes (Dawar *et al*., 2007).

Vaccine acceptance: Underestimates of both the level of risk and the severity of HPV-associated disease may be barriers to vaccine acceptance. Hence, vaccine acceptance can be maximized by effectively communicating the risks associated with HPV infection and the benefits of vaccination. Educational initiatives targeted towards parents and the public in general are invaluable in fostering positive attitudes towards vaccination.

More than 70% of the parents of girls between the ages of 8 and 18 years, who were surveyed in a national study showed an intention of having their daughters receive the HPV vaccine in school based, publicly funded vaccination programs for girls 11 and 12 years of age. Just over 20% of the parents of girls expressed concerns about the influence of the HPV vaccine on sexual behaviour (Ogilvie *et al*., 2007).

CONCLUSION

A recent advance in the history of medicine was the development of 2 HPV vaccines that prevent a person from becoming infected with HPV. Since prolonged and persistent infections with the oncogenic genotypes of HPV are the main reason behind the development of cervical cancer, preventing infection with these subtypes is the most effective way to eradicate cervical cancer.

Educational initiatives targeted mainly towards parents to have their children immunized against HPV at an early age are of immense importance. The vaccine will be of no use if the person receiving, it has already been infected with the HPV subtypes that the vaccine is expected to protect the person from. It is thus, of crucial importance that the vaccine is given before a person is exposed to HPV. The vaccine is also more efficacious at a young age; in fact, a given dose induces higher antibody titres and therefore, a stronger immune response in young females than in adult ones.

The 2 HPV vaccines that have been manufactured, Gardasil and Cervarix, protect against HPV-16 and HPV-18, which are responsible for the majority of cervical cancers. Yet, some 30% of cervical cancers are caused by other

HPV types that these vaccines do not protect against. For this reason, cytology screening must still be done on women who have been vaccinated against HPV-16 and HPV-18, because they may still develop cervical cancer. The incidence of cervical cancer will be reduced further if not only women but also men are immunized against HPV. Once again, developing countries pose a problem because people living here may not afford to take the vaccine.

The introduction of the HPV vaccine will hopefully once more lead to a substantial decrease in cervical cancer incidence, following the decrease that was observed on the introduction of the Papanicolaou smear. A greater initiative should be undertaken, however, as regards developing countries, which will probably be the last ones to afford to take the vaccine; countries, which also lack organized screening programmes, lack education about the disease and lack appropriate treatment once the disease manifests itself.

REFERENCES

Baud, D., F. Ponci, M. Bobst, De P. Grandi and D. Nardelli-Haefliger, 2004. Improved Efficiency of a Salmonella-based vaccine against human papillomavirus type 16 Virus-Like Particles Achieved by Using a Codon-Optimized Version of L1. J. Virol., 78: 12901-12909.

Bonfiglio, T.A. and M.H. Stoler, 1993. The Pathology of Cancer. In: (Rubin, P.) 7th Edn. Clinical Oncology. A Multidisciplinary Approach for Physicians and Students. New York: W.B. Saunders Company, 3: 29-40.

Castle, P.E., D. Solomon, M. Schiffman and C.M. Wheeler, 2005. Human papillomavirus Type 16 Infections and 2 year Absolute Risk of Cervical Precancer in Women with Equivocal or Mild Cytologic Abnormalities. J. Natl. Cancer Inst., 97: 1066-1071.

Craig, P. and D. Lowe, 2003. Non-neoplastic conditions of the cervix. In: H. Fox and M. Wells. (Eds.). Obstetrical and Gynaecological Pathology, 1 (7): 273-95. United Kingdom: Churchill Livingstone.

Dawar, M., S. Deeks and S. Dobson, 2007. Human papillomavirus vaccines launch a new era in cervical cancer prevention. CMAJ., pp: 177.

Emeny, R.T., C.M. Wheeler, K.U. Jansen, W.C. Hunt, T.M. Fu, J.F. Smith, S. MacMullen, M.T. Esser and X. Paliard, 2002. Priming of Human Papillomavirus Type-11-Specific Humoral and Cellular Immune responses in College-Aged Women with a Virus-Like Particle Vaccine. J. Virol., 76: 7832-7842.

Geipert, N., 2005. Vaccinating Men for HPV: New Strategy for Preventing Cervical Cancer in Women? J. Natl. Cancer Inst., 97: 630-631.

Honey, K., 2006. HPV vaccine gets a shot in the arm. J. Clin. Invest., 116: 3087.

IARC, 1995. Working Group on the Evaluation of Carcinogenic Risks to Humans: Human Papillomaviruses. Lyon, France. Monographs on the Evaluation of Carcinogenic Risks to Humans. Human Papillomaviruses. Vol 64. United Kingdom: World Health Organization, International Agency for Research on Cancer. Image: Cases of HPV-induced cancers graph. png-Wikipedia, the free encyclopedia website. http://en.wikipedia.org/wiki/Image:Cases_of_HPV_cancers_graph.png.

Khan, M.J., P.E. Castle, A.T. Loinez, S. Wacholder, M. Sherman, D.R. Scott, B.B. Rush and A.G. Glass, M. Schiffman, 2005. The elevated 10 year risk of Cervical Precancer and Cancer in Women with Human Papillomavirus (HPV). Type 16 or 18 and the Possible Utility of Type-Specific HPV Testing in Clinical Practice. J. Natl. Cancer Inst., 97: 1072-1079.

Lippman, S.M. and W. Ki Hong, 2002. Cancer Prevention Science and Practice. Cancer Res., 62: 5119-25.

Liu, D.W., Y.P. Tsao, J.T. Kung, Y.A. Ding, H.K. Sytwu, X. Xiao and S.L. Chen, 2000. Recombinant Adeno-Associated Virus Expressing Human Papillomavirus Type 16 E7 Peptide DNA Fused with Heat Shock Protein DNA as a Potential Vaccine for Cervical Cancer. J. Virol., 74: 2884-2888.

Lowy, D.R. and J.T. Schiller, 2006. Prophylactic human papillomavirus vaccines. J. Clin. Invest., 116: 1167-1173.

Mandic, M. and T. Vujkov, 2004. Human papillomavirus vaccine as a new way of preventing cervical cancer: a dream or the future? Ann. Oncol., 15: 197-200.

Mayor, S., 2005. Cervical cancer jab in a year. BMJ., 331: 864.

Ogilvie, G.S., V.P. Remple, F. Marra, S.A. McNeil, M. Naus, K.L. Pielak, T.G. Ehlen, S.R. Dobson, D.M. Money and D.M. Patrick, 2007. Parental intention to have daughters receive the human papillomavirus vaccine. CMAJ., 177: 1506-1512.

Onon, T.S., 2003. Vaccines. In: Prendiville, W., J. Ritter, S. Tatti and L. Twiggs (Eds.). Colposcopy Management Options. Ireland: Saunders, 13: 105-122.

Peng, S., H. Ji, C. Trimble, L. He, Y.C. Tsai, J. Yeatermeyer, D.A.K. Boyd, C.F. Hung and T.C. Wu, 2004. Developmant of a DNA Vaccine Targeting Human Papillomavirus Type 16 Oncoprotein E6. J. Virol., 78: 8468-8476.

Palefsky, J., 2002. What your doctor may not tell you about HPV and abnormal Pap Smears. New York: Warner Books.

Rambout, L., L. Hopkins, B. Hutton and D. Fergusson, 2007. Prophylactic vaccination against human papillomavirus infection and disease in women: A systematic review of randomized controlled trials. CMAJ., 177: 469-479.

Tanne, J., 2006. CDC recommends expensive vaccine for all girls aged 11-12. BMJ., 333: 114.

Underwood, J.C.E., 2004. Carcinogenesis and neoplasia. In: Underwood, J.C.E. 4th Edn. General and Systematic Pathology. United Kingdom: Churchill Livingstone, 11: 223-262. University of Rochester Medical Center. http://www.urmc.rochester.edu/GEBS/faculty/Images/vlps.jpg.