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Review Article

Human Papillomavirus and Cancer-Immunological Consequences of MHC Class I Down-Regulation

Gholam Hossein Ashrafi*[1], Dhaya Perumal[2]

1. Cancer Study Group, SEC Faculty, Penrhyn Road, Kingston University London, Kingston Upon Thames, UK.
2. School of Pharmacy and Chemistry, SEC Faculty, Penrhyn Road, Kingston University London, Kingston Upon Thames, UK.

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Few studies have been conducted on the causative factors associated with the development of cancer. Infection by high risk human papillomaviruses (HPVs) have been implicated as causative agents in a variety of cancers. HPV is capable of evading immune system and establishing persistent infections. Prolonged infection and lesion maintenance are associated with higher risk of neoplastic progression. Hence, curtailing the ability of the virus to escape host immunosurveillance should reduce this risk by accelerating resolution of infection and lesion progression. One of the potential effectors of HPV escape from host immunosurveillance is the E5 oncoprotein, which we have shown to down-regulate surface major histocompatibility complex class I (MHC I), without apparent effect on non-classical MHC. These effects would interfere with both cytotoxic T lymphocyte (CTL) killing of the virally infected cells, and with the natural killer (NK) cell illumination of infected cells.
In this review we address mechanisms of immunomodulation by papillomavirus and discuss our current findings on the association of HPV and cancers.

Keywords: Human papillomavirus (HPV), MHC I, cytotoxic T lymphocyte (CTL), cancer

Cancer is a global burden responsible for over 7.5 million deaths a year (1) and is thus a leading cause of death, second only to cardiovascular disease (2, 3). In the UK alone, there were over 320,000 newcases diagnosed in 2010 and approximately 157,000 deaths (4).

Despite the medical importance of cancers, few studies have been conducted on the causative factors associated with the development of cancers. Although it is well-known that multiple risk factors are associated with the development of cancer, the initiating cause has not been identified.

Infectious agents have been implicated, either as direct carcinogens or as promoters. Viral infections, in particular, human papillomaviruses (HPVs) are recognised as carcinogenic agents in humans, and are responsible for a significant share of the global cancer burden (1, 5, 6).

Although the body is working to get the infection under control, HPVs disturb cutaneous and

*Correspondence : Cancer Study Group, SEC Faculty, Penrhyn Road, Kingston University London, Kingston Upon Thames, UK, KT1 2EE. E-mail:h.ashrafi@kingston.ac.uk

mucosal epithelial cells of the anogenital tract, which can lead to a variety of diseases with a range of severities. The mildest forms of HPV disease are benign epithelial hyperproliferative lesions, such as genital warts (condylomas) and low grade intraepithelial neoplasia (CIN1). These lesions can regress even without treatment (7), due to cell mediated immune responses (8). In a few cases however, the response is lacking or ineffective and the lesions persist, recur or progress to invasive cancer. Typically, CIN1 has been reported to progress to high grade disease (CIN3) and invasive cervical cancer (9, 10). Hence, the molecular associations of the immune response, HPV infection and the development of cancer are an area of continuing interest.

Here, we discuss the mechanisms of host immune suppression by HPV genome and explore the importance of this immune evasion in the establishment and persistence of papillomavirus infections and neoplastic progression of premalignant lesions to squamous carcinoma. Considering the broad interest in HPV vaccines, it is very important to verify the prevalence of the various HPV types worldwide, especially the high-risk ones. Therefore, we further review our current findings on distribution of HPV genotypes in breast cancer and cervical cancer.

Human papillomavairus, immune response and immune evasion

Papillomaviruses (PV) are a large family of small DNA viruses that infect cutaneous or mucosal epithelial cells (11). HPVs are large family of common viruses that infect epithelial surface (skin, genital) and cause benign lesions (known as warts or papillomas). Although HPV infections are normally cleared by the immune system, albeit after a long delay period, persistence of HPV can cause a progression to malignant disease under appropriate environmental conditions. For example, infection of the cervix with “high risk” HPV types 16 and 18 has previously been reported to be the initiating event in cervical cancer (12, 13). Long term viral persistence is necessary for malignancy and additionally requires avoiding immune attack and clearance (14-16).

There is much circumstantial evidence that the host cell mediated immune response plays an important role in the control of HPV infection. Spontaneous regression of HPV induced lesion is associated with an inflammatory response and infiltration of lymphocytes to the affected area (8). Furthermore, HPV induced lesions are far more common in individuals who are immunosuppressed following transplant surgery (17, 18) or if infected with HIV (19). Failure to clear the HPV infection and consequent viral persistence are crucial factors for many months before the onset of an immune response, and one of the crucial unresolved questions in the PV field is the delay of the immune response in the eradicating the virus/ viral infected cells even in healthy immunocompetent hosts.

One of the reasons for poor immunological recognition of HPV infected cells is the non-lytic nature of the infection, and consequent lack of inflammation, coupled with the epithelial confines of the virus life cycle (20, 21). However, this “passive” immunoscape is not the only cause of the delayed immune response. There is also a growing awareness that the virus actively fights the host immune system: the three early viral oncoproteins, E5, E6 and E7 are all capable of interfering with the host cellular immune response. E6 and E7 disrupt the interferon (IFN) pathway or affect the expression of TAP (22, 23), while Ashrafi et al. have found that

E5 down-regulates surface MHC class I by retaining it in the Golgi apparatus (Fig 1 and 2) (16, 24).

Down-regulation of MHC class 1 has been observed with E5 from different PVs, including functions of E5 are conserved between the PV and HPV types (14). E5 is a small hydrophobic protein

Down-regulation of MHC class I by bovine papillomavirus E5 oncoproteins

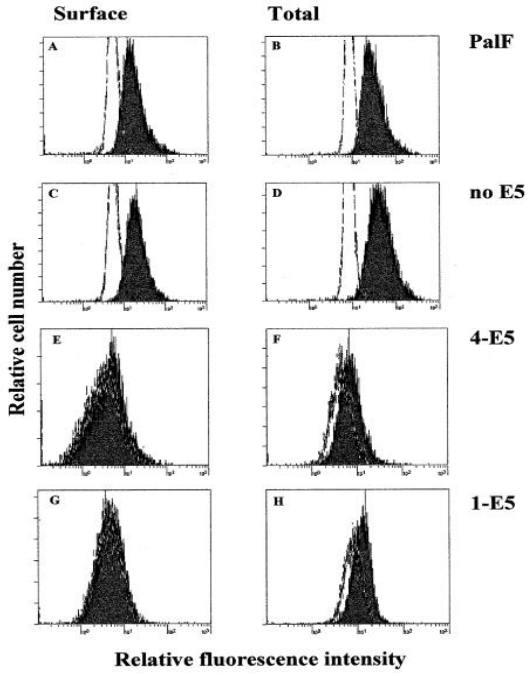

Figure 1. FACS profiles of MHC I expression in parental and transformed PalF cells. Control PalF cells (**A,B**), transformed control cells (**C,D**), 4-E5 transformed cells (**E,F**) and 1-E5 transformed cells (**G,H**) were stained with mAb IL-A19 and analysed by flow cytometry. Surface MHC I was measured in intact cells (**A, C, E, G**) and total MHC I was measured in saponin-permeabilized cells (**B, D, F, H**) black histograms (24).

(83 amino acids in HPV-16), located in the cell endomembranes, which are expressed early during the infectious viral cycle. It interacts with 16k subunit c of the vacular H^+ V-ATPase, the pump that maintains an acidic pH in the membrane compartments, and is so doing impedes the proper function of the pump with increased alkalization of the endosomes and Golgi apparatus (25).

MHC class I (HLA I in humans) presents antigenic peptides to CD8 positive cytotoxic T-lymphocytes (CTL), allowing recognition and killing of virally infected cells. Within the infected cell, the virus proteins are degraded to short chain peptides, typically 8-10 amino acids long, by proteasomes and other cytoplasmic proteases (21). These epitopes (peptides) are transported through the Golgi apparatus to the cell surface where it is presented to activate specific CTL. The CTL are a-ble to kill the infected cell through apoptosis mediated either by granulate exocytosis (perforin and granzymes) or by Fas-Fas ligand interaction. HPV-specific CTL has previously been detected in cervical cancer and CIN3 patients, but at low frequency in comparison to CTL specific for other human viruses (26, 27).

CD4 positive T helper lymphocytes (Th) recognize foreign antigens in association with MHC class II molecules. MHC class II molecules are found predominantly on professional antigen presenting cells (APC) such as dendritic cells, macrophages and B cells but can be expressed on epithelial cells (the target for HPV) by IFN treatment. The cells secrete cytokines that allow proliferation and maintenance of CTL and also activate dendritic cells for antigen presentation and B cells for antibody production. The cells are likely to have an important role in cell mediated immunity against HPV. Indeed HPV-16 specific the responses have been detected in patients with persistent HPV infection and in healthy individuals (28, 29).

MHC class I down-regulation by E5 would help in the establishment and persistence of virus infection, by potentially allowing the infected cell to evade killing by CTL and thus increasing the likelihood of recurrent genital warts and/ or cancer.

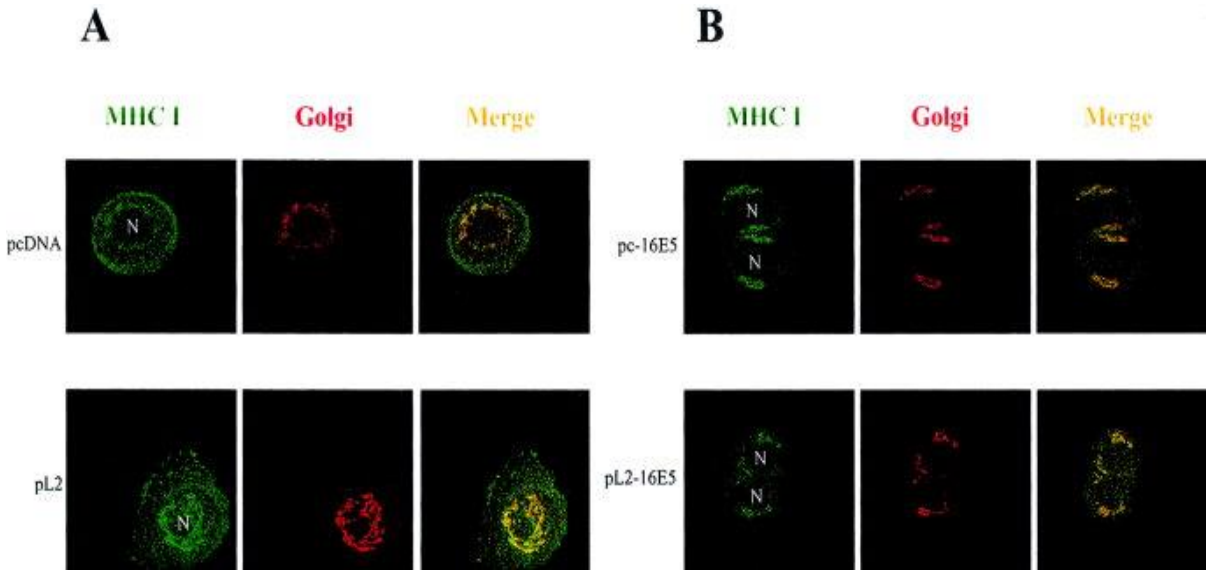

Figure 2. HLA class I is retained in the Golgi apparatus in HPV-16 e5-Expressing cells. HaCaT cells carrying empty vectors or expressing E5 (at least 3 lines of each were stained with mAb W 6/32 (anti-HlA calss I) and mAb 4A3 (antigoglin GM130) and analyzed using confocal microscopy. Representative cells are shown. (A) Control HacT cells carrying either pcDNA or PL2 Empty vector. (B) HeCAT cells expressing HPV-16 E5 in either pcDNA (pc-16E5) or pl2 (pl2-16E5).Legend: N,nucleus (16).

It is interesting to note that E5 has been reported to additionally inhibit the Fas receptor (30) and HLA II (31), strongly indicating that E5 can play a major role in several aspects of the cell mediated immune response.

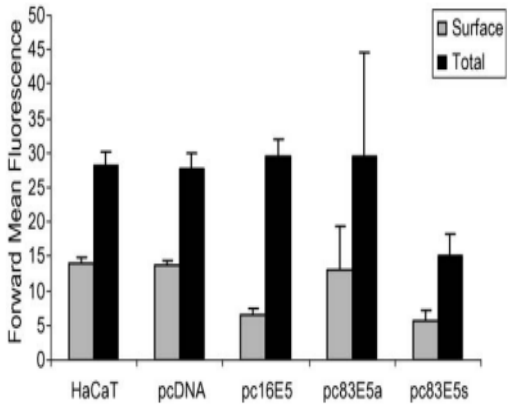

Figure 3. HPV-83 E5 down-regulates MHC class I. Parental HaCaT cells, cells harboring empty vectors (pcDNA), pc16E5, pc83E5s (83E5 in sense orientation) or pc83E5a (83E5 in antisense orientation) were analyzed for expression of total and surface MHC class I by flow cytometry with mAb W6/ 32. The average meanfluorescence was calculated from the flow cytometric analyzes of three duplicate measurements from at least two clones of each cell line. The background reading of cells stained with no primary antibody and only secondary antibody was 0.4 in all cases. Standard deviation is shown (14).

Although MHC I down regulation is an efficient mechanism to avoid CTL-mediated immune clearance, the total absence of surface MHC I render cells more susceptible to natural killer (NK) cell attack. Human NK cells express multiple receptors that interact with HLA class I molecules, including killer cell immunoglobulin-like receptors (KIRs) that predominantly recognize classical HLA class I, including HLA-C, and the C type lectin superfamily of receptors that specifically interact with the non-classical class I molecule HLA-E. Recognition of the class I molecules by their inhibitory receptors inhibits NK-mediated cell lysis, which would occur in the absence of HLA-C/ E. Accordingly, certain viral proteins, including HIV Nef and the US3/ UL40 proteins of CMV, have evolved to selectively down-regulate HLA-A and-B, the main presenters of peptides to CTLs, but not HLA-C or-E, and are therefore capable of avoiding both CTL and NK cell killing (32, 33).

Ashrafi et al. (24) have been the first to report that E5 protein of human papillomavirus (HPV), which is localized in the GA, down-regulates classical

HLA (HLA-A & B) and does not affect the non-classical HLA complex thus potentially allowing the cell to escape the killing by CTL and also NK cells (16).

High risk HPV types and cancer

Despite the medical importance and high incidence rate of cervical cancer, there is a lack of information on the incidence of HPV genotypes and their distribution in cervical cancer patients in different populations. Our recent investigation on distribution of HPV genotypes in cervical tumour tissue of patients revealed the presence of HPV-45 and HPV-39 in patients with cervical cancer (13).

Regardless, while the body is working to get the infection under control the virus can spread through sexual and skin-to-skin contact. HPVs have also been found to cause close to half of vaginal, penile, anal and oral cancers (12). These findings suggest that HPV DNA might be transported from the original infection site to other organs, and may be responsible for the development of cancer in various organs.

Studies on HPV role in breast carcinogenesis have generated controversy and it is still not clear whether HPV is present in breast tumors (34). Our unpublished findings have shown the evidence for the presence of high risk HPV types viral DNA other than 16 and 18 in freshly obtained human breast cancer tissue and now provides a solid basis to advance research in a crucial health imperative affecting women.

This early findings strengthen the association of HPV and breast cancer and will allow us to address important questions on the causative agents of breast cancer. However, further research is is needed to understand whether HPV plays any role in the pathogenesis of breast cancer. The knowledge acquired will lead to better understanding of risk factors other than those established to date.

Conclusions

Our findings on HPV and cancer provide valuable baseline data for future assessment of the impact of current prophylactic vaccination programs that is protective against the two most common oncogenic types of HPV found in HPV related cancers, HPV-16 and HPV-18, but not against other high-risk mucosal HPVs, HPV-39 and 45, as reported in our study on HPV and cervical cancer (13) and also new high risk HPV types detected in our investigation on the association of HPV and breast cancer (Ashrafi et al. unpublished data). Cancer prevention, viral carcinogenesis and cancer are rapidly developing sectors of this field and the prospect of investigating the use of wide spectrum vaccines to prevent HPV related cancer is very appealing.

Conflict of interests

The authors declared no conflict of interests.

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