HPV and carcinogenesis

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SUMMARY

The HPV's mechanism of carcinogenesis is not completely understood. The possibility of evolving into direction of malignancy depends on the type of virus, the synergic action with different physical, chemical and biological agents, the genetic constitution of the host and the immune defense mechanism of the host, all of which are able to modify the course of HPV infection.

KEY WORDS

HPV infection, DNA genotypes, carcinogenesis

Introduction

The human papillomaviruses (HPV) are small viruses with double stranded DNA that have a particular tropism for the epithelium inducing its proliferation. It is believed that the HPV enters the body after slight trauma to the epithelium and needs terminally differentiated epithelial cells for replication (1).

Up to now 100 (2) different genotypes have been recognized, of which one small group has been identified as a causing agent for certain types of tumors in several epithelia. It is the number one cause of cervical carcinoma (3).

The DNA of the HPV can persist in the infected cell in episomic or extrachromosomic form or can be incorporated into chromosomes of the host cell. Consequently, the derived cells will also carry within their genetic material the genetic material of the virus, responsible for the cellular transformation.

The HPV genome contains a double-stranded circular DNA of about 7900 base pairs that can be functionally divided into two regions:

1. LCR (Long Control Region) - necessary for the regulation of the genic expression and for the DNA replication.

2. **ORF** (Open Reading Frames) - that can be divided into the Early Region, necessary for the replication, cellular transformation and for the control of viral transcription and the Late Region that codes for the capsid proteins that comprises the outer protein coat of the virus (1).

Within the Early Regions (E), it is possible to distinguish different genes with specific functions:

- E1 and E2 have an important role in viral DNA replication. The E2 participates in the regulation of LCR transcriptions, and decreases the expression of E6 and E7.

- E3, whose function is still not known
- E4, which codes one family of small proteins involved in the transformation of the host cell producing alterations of the mitotic signals and interacting with the keratin which becomes destabilized.
- E5 interacts with the growth factor's receptors and stimulates the cellular proliferation. It also decreases intercellular communication with the aim to isolate the transformed cells. It also stimulates the expression of E6 and E7.
- E6 acts as an oncogene, stimulating the growth and transformation of the host cell by the inhibition of protein p53's normal oncosuppressor function.
- E7 acts as an oncogene, inducing cellular proliferation by inhibition of the protein pRb, p107 and p130.
- E8, whose function is still not known.

Within the Late Region (L), it is possible to distinguish:
- **L1** which codes for major capsid protein and can form virus-like particles.
- **L2** which codes for minor capsid protein.

The oncogenic mechanism

The HPV's mechanism of carcinogenesis is not completely understood. HPV can produce immortality in keratinocytes and acts alone even if different cofactors, still not completely located, are necessary for malignant conversion.

The possibility of evolving into direction of malignancy depends on the type of virus, the synergic action with different physical, chemical and biological agents, the genetic constitution of the host and on the immune defense mechanisms of the host, all of which are able to modify the course of HPV infection.

In the case of high risk HPV infection and under favorable conditions, the viral genome is integrated into the host genome which is the necessary event for the keratinocytes immortality (4). During this process of integration the circular form of viral genome breaks at the level of the E1 and E2 regions, never at the level of the E6 or E7 region. Different studies have shown that the integrated part of the genome corresponds to E1, E6 and E7 while the regions from E2 to E5 are lost and are not transcribed in the tumors. The loss of E2 during this process of integration produces the loss of E6 and E7 control. Therefore, the sequences E6 and E7 are directly involved in the cellular cycle by inhibiting the normal functions of p53 and pRb respectively (5). The protein p53 is known as the "genome's guard" and in the case of DNA damage, the p53 can provoke the arrest of cellular division and assure the time necessary for DNA repair (6). If damage can not be repaired, p53 is able to induce the programmed cellular death and prevent the propagation of DNA damage in subsequent generations of cells. In the case of other types of tumors p53 is usually mutated and acts as a real oncogene. In the case of HPV infection, E6 suppresses the properties of p53 **gene product** achieving the functional equivalent of the two hits required to knock out both alleles of a tumor suppressor gene (7). The mutations of p53 are normally not found. The E7 protein interacts with retinoblastoma protein (pRb), which is the crucial factor for the cellular cycle control. This interaction causes the release of the transcription factor E2F, which is now free to act and can stimulate the cellular division. E7 is also able to bind and inactivate the proteinkinase inhibitors p21 and p27 and can interact with different proteins whose significance has still not been determined.

E6 and E7 can cooperate with cellular oncoproteins like *ras* and *myc* which enables the virus to act at the level of growth factors and cellular and nuclear metabolism producing oncogenic cells. E6 and E7 can provoke directly DNA mutations of the host cell, probably by causing alterations of DNA repair mechanisms. This means that certain types of HPV are able to cause malignant lesions even without the action of other cofactors.

The exact role of the immune response against high risk HPVs is not completely clear. HPVs are obligatory intraepithelial pathogens that replicate at the superficial layers of the mucosa and epidermis where the cells are more differentiated. Both types of immune response (antibodies and cell mediated) have been demonstrated in humans. Cell-mediated immunity plays a crucial role in controlling HPV infection.

The antibodies against HPV can be of the type IgA, IgM or IgG reaching maximum levels 6 to 12 months after the beginning of the infection. There is an increased prevalence of antibodies against proteins E7 and E4 in patients with cervical intraepithelial neoplasia and with cervical carcinoma. It is possible that in the future the measuring of the antibodies against E7 will become a marker to assess the response of a specific therapy. The presence of antibodies against E4 is associated with viral replication and is believed to coincide with the first host's contact with HPV (8). The regression of HPV lesions is associated with a characteristic histologic response with participation of T lymphocytes and activated macrophages (cellularly mediated). In the case of immunosuppressed patients the possibility of high risk infections is increased because of the lack of immune response, the oncogenic effect of the drug administration, as well as chronic antigenic stimulation.

HPV related neoplasias

The idea that cervical carcinoma can be related to sexual activity and to infective agents was postulated in 1842 (9). Subsequently, many sexually transmitted in-

fective agents have been suspected as responsible for this type of neoplasia. In 1977, by means of cytologic, hystologic and colposcopic studies, the first evidences of the involvement of HPV in cervical cancer (CC) were obtained. In 1983, Syrjänen identified HPV viral antigens in 50 % of the cases of cervical displasia. In the following years, different types of HPVs have been identified in correlation with different neoplasias (10). Up to now high risk HPVs have been known to be involved in the following diseases:

1. Epidermodysplasia verruciformis (EV)

This is a rare, lifelong, autosomal recessive hereditary disorder affecting the skin characterized by dysfunction of cell-mediated immunity. The disease usually begins in infancy or early childhood with development of various types of warts and plaques involving mostly sun-exposed areas of the skin. Different types of HPV have been identified in the lesions: HPV 3, 5, 8, 9, 10, 12, 14, 17, 20, 21, 23, 25, 28, 38, 47 and 49. The combination of different HPV types, such as 3, 5, 8, 14 and 17, immune deficiency and solar exposure result in high risk for multiple skin cancers (principally squamocellular type) in these patients (11,12). Cutaneous carcinomas, developing in about half of EV patients, typically appear during the fourth and fifth decades of life and are usually associated with the oncogenic HPV 5 (13) and HPV 8. Certain authors believe that EV is not a typical HPV provoked neoplasia because genetic disposition is involved.

2. Cervical intraepithelial neoplasia (CIN) and invasive cervical carcinoma (ICC)

In the case of cervical neoplasia of the uterus the presence of HPV is greater than or equal to 95 %. Different types of HPV have been identified: 16, 18, 31, 33, 35, 39, 42, 43, 44, 51, 55, 58, 72 and 73. *Types 16 and 18 are most clearly shown to be a human carcinogen.* Among other anogenital HPV types 6, 11, 26, 27, 30, 35, 39, 40, 45, 59, 61, 62, 64, the *epidemiological evidence is strongest for HPV 31 and 33* (14). In the majority of cases the presence of HPV alone is not sufficient for the development of neoplasia and different cofactors have been identified:

- tobacco, probably by tar deposits that interfere with the cervical physical barrier and with local Langerhans cells;
- other sexually transmitted diseases (e.g., HIV, herpes virus, *Chlamydia* species);
- conditions of temporary immunodeficiency, such as pregnancy, the use of contraceptive drugs, steroid treatment; or permanent immunodeficiency, as in the case of leukemias, lymphomas, AIDS, renal grafts, etc. In the cervical smear the presence of HPV is three times more frequent during the pregnancy than in non-pregnant women;
- alterations of hormonal status;
- beta-carotene deficiency;
- repeated local traumas and promiscuity, which increase the statistical probability;
- some modalities of sexual behavior (10).

3. Vaginal intraepithelial neoplasia (VAIN) and vaginal carcinoma (VC)

It is not frequent when compared with cervical neoplasia and in many cases it represents the propagation of cervical neoplasia. In this type of neoplasia HPV is present in 50 % of cases and the types usually identified are 16, 18 and 31. However the routine diagnostic procedures have not been directed to intraepithelial neoplasias other than CC.

4. Vulvar intraepithelial neoplasia (VIN) (Bowenoid papulosis, erythroplasia of Queyrat and vulvar carcinoma)

In these conditions HPV is present in more than 50 % of the cases, usually type 16. Patient history usually reveals the previous presence of condyloma (15). Recent studies suggest that in erythroplasia of Queyrat, in contrast to other genital neoplasias, a co infection with HPV type 8 and HPV type 16 occur. The presence or absence of HPV type 8 might help to distinguish between erythroplasia of Queyrat and Bowen's disease.

5. Penile carcinoma (including Bowenoid papulosis)

HPV can be identified in 50 % of cases and is strongly associated with type 16. Patient history is frequently positive for the presence of condylomas. Buschke-Lowenstein syndrome or giant condylomatosis is associated with types 6 and 11, which are considered as virus types with low risk.

6. Anal (AC) and perianal carcinoma (PC)

HPV has been found in more than 70 % of cases. The identified types of HPV correspond to those found in the cervical neoplasia. AC and PC are much more common in immunosuppressed patients like HIV-infected individuals.

7. Oropharyngeal carcinoma (EC)

HPV DNA has been found in 20 % of tumors localized at the tongue and tonsillas and corresponds to the types identified in the anogenital lesions.

8. Esophageal carcinoma (EC)

For this type of carcinoma the results are still not clear. The role of infected agents, among them of HPV,

is already suspected and some authors have isolated HPV in 15 % - 30 % of the biopsy specimens. HPV 73 was identified in EC by some authors (16).

9. Non-melanoma skin cancers (basal cell carcinoma-BCC and squamous cell carcinoma-SCC)

The presence of HPV-DNA is demonstrated in 90% of cutaneous SCC from renal allograft recipients. These HPV types (20, 23 and 38) are all related to the epidermodysplasia verruciformis group. The relation between infection with certain types of HPV and the development of non-melanoma skin cancers in immunocompetent patients has still not been explained and may be just casual or the virus can be a real etiologic agent. One recent study with 61 immunocompetent patients suggests that the occurrence of HPV-DNA in BCC does not reflect a major etiologic role of HPV in this cancer (17).

The role of HPV in non-melanoma skin cancer carcinogenesis remains speculative (18).

10. Melanoma

The HPV could be detected in melanoma biopsy specimens, but it has not a role in inducing the development of this tumor. HPV may be correlated with rapid melanoma progression because may act as a cofactor (19).

Conclusion

The evidence of association between certain tumors and HPV infection today is indisputable. In the case of anogenital tumors different types of HPVs have been identified. Of all the sexually transmitted diseases, condylomas have the highest frequency in the developed world. Different studies are trying to determine the cancer risk after HPV infection, with the results varying from 6 % to 33 % and a time interval from 1.7 to 2.7 years for intraepithelial neoplasias and 6 years for invasive tumors.

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