THE IMPACT OF HUMAN PAPILLOMA VIRUS (HPV) INFECTION ON THE DEVELOPMENT OF CERVICAL NEOPLASIA

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Abstract . The genesis of uterine cervix carcinoma has as central etiology the active infection of human papilloma virus (HPV), especially high oncogenic risk subtypes. However, this is a necessary, but not sufficient cause of virtually all cases of cervical cancer worldwide. At present, the proportion of cervical carcinomas attributed to HPV infection is estimated at 99%. It is considered that the pathogenesis of cervical carcinoma is the result of the proliferation of one or, at most, a few HPV-infected cells. Invasive cervical cancer arises in cervical intraepithelial neoplasia, which in turn develops preferentially in squamous metaplasia of certain limited areas. These areas represent the most important morphologic characteristic in cervical intraepithelial neoplasia. It is still unknown the precise mechanism for the development of separate fields in HPV-related intraepithelial neoplasia and the variable susceptibility of reserve cells for different HPV genotypes. The goal of any cervical cancer screening test is to identify women who are at risk of cervical cancer development and to reassure others that do not belong to this category. Cervical cytology (Pap smears) were the primary screening tests. More recently, carcinogenic HPV DNA testing has been included as adjunctive test or as a primary screening test based on the central role of carcinogenic HPV infection in the development of cervical cancer. New biomarkers, including those that measure the interaction of host and virus, are being considered either as stand-alone molecular assays or in conjunction with cytology or carcinogenic HPV DNA testing to improve its sensitivity or specificity, respectively. .Profilactic HPV vaccination of women who are sexually active may provide protection against HPV-16 or HPV-18 infection which may lead to cytological abnormalities, precancer or cancer.

INTRODUCTION

Through the last two decades it was produced an explosion of informations about cervical carcinoma etiology and his precursor lesions. Presently, it is accepted that either invasive squamous carcinomas and cervical adenocarcinomas, as correspondent precursor lesions, are determined by specific types of HPV which infects the anogenital tract. The genesis of uterine cervix carcinoma has as central etiology the active infection with human papilloma virus (HPV) especially high oncogenic risk types. At present, the proportion of cervical carcinoma attributed to HPV infection is estimated to 99%. Many observations have indicated the importance of immune response in papilloma virus infection. Antibodies against capsid proteins of different types of HPV were anterior studied, using fusion proteins bacterian expressed or chemically synthesized peptides. As an essential condition to these studies, it has to be detected infectant HPV type. Until recently, the detection of HPV type in a certain tissue used hybridization arrays with nucleic acids. As a more specific and sensitive method for HPV DNA amplification it was developed PCR (polymerase chain reaction), either for general detection and for identification of HPV types, particular in genital infections. A difficulty of this procedure is the problem of cuantification, the distinction between latent subclinic infection and evident clinic lesions. As an alternative to hybridization and PCR, the immunologic detection of viral capsid antigens can be used for the diagnostic of productive HPV infections.

RESULTS AND DISCUSSIONS

Human papilloma viruses in correlation with squamous cervical epithelium. HPV role in cervical carcinoma genesis

Human papilloma viruses (HPV) represent DNA viruses that infect skin and mucosal epithelial cells, inducing proliferative benign and malignant lesions. *Infection with a high risk HPV represents the major cause in the development of uterine cervix carcinoma,* the secondly wide world prevalent type. In late '70th , based on theoretical reasons, zur Hausen (1) suggested the existence of the possibility of an association between HPV and cervix carcinoma. Koss and

Durfee (2) had introduced, in 1956, the term of koilocytic atypia in order to describe abnormal squamous epithelial cells characterized by a prominent perinuclear vacuolization (koilocytes); these cells were detected in Babes-Papanicolau cytological tests in patients with cervical dysplasia and invasive cervical carcinoma. In 1976, Meisels and co. (3) published papers in which they suggested that cells of condilloma acuminate that contain viral particles compatible with HPV in electron microscopy were cytologically identical with koilocytes described by Koss. In short time afterward, several research groups had detected viral particles by electron microscopy or capsid HPV proteins, by immunohistochemistry, in low grade squamous intraepithelial lesions (LSIL).

Molecular techniques contribution in elucidation of HPV role in cervical carcinogenesis

By molecular techniques applied in the study of cervical neoplasia, rapid progresses were made in understanding relatioships between HPV and cervical cancer. Zur Hausen and co. were the first to isolate new types of HPV in anogenital lesions and had demonstrated that specific types of HPV DNA may be identified by Southern blot hibridization in majority invasive squamous cervical carcinomas and in a great number of precursor lesions (4).

Structural characteristics of HPV. Subtypes, localisation

Papilloma viruses are classified as members of A Papovaviridae family, that includes simian virus 40 (SV 40), polyoma virus, and also papilloma viruses. All the members of Papovaviridae family are tumoral viruses double-stranded DNA, that are not similar as dimensions and possess only limited DNA homologous sequences. Characteristics HPV aspects that sepparate them from the other members of the Papovaviridae family are: a double-stranded DNA of approximately 8000 pairs of bases in length, a noncapsulated virion that measures 45-55 nm in diameter and a icoshaedric capsid composed of 72 capsomeres. HPV are widely found in nature. They are of canine, bovine, avian, rabbit, dear, and human types. HPV capsid protein has antigenic similitudes with that of bovine papilloma virus (BPV), and this characteristic is used in polyclonal sera production. Differentiatingly from other numerous viruses that posses capsids with different antigenic structures, HPV capsid proteins are highly conservated and antibodies directed against capsid BPV proteins have a crossing reaction with HPV (5). Consequently, specific types of HPV can not be serologically identified (serotypes) and DNA sequence is used to classify different viral types (genotypes). Recently, DNA sequences analysis was used to classify papillomaviruses. In human species, 85 types of papilloma viruses were characterized and completely sequenced and more than 120 presumed new types were partially characterized. Although different HPV types are almost similar as structure, they have a significant specificity correlated with the anatomical location of infected epithelia and with type of lesion in the infectious site (6). Supplementary, there are also subtypes or variants of specific type, as HPV-16. In order to be considered a subtype or a variant, a virus should be different with 2-5% from the originally isolated type.

HPV infection tropism - specificity correlated to viral type

Papilloma viruses represent epitheliotropic viruses that infect predominantly the skin and mucous membranes and produce characteristic proliferations at the infected site. These benign proliferations or papillomas have the capacity to transform in a malignant fashion in certain conditions. In human species, HPV infections appear on the skin, mucosae, conjunctiva, oral cavity, larynx, trachea, bronhy conducts, esophagus, urinary bladder, anus, and genital tracts, in both genders. HPV are difficult viruses regarding the required conditions for their development, replying only in the nucleus of infected cells. Moreover the species- specificity, HPV are relatively tissue-specific and situs-specific. For example, HPV-1 preferentially infects squamous

epithelium of plants of the feet, producing plantar verrucae, meanwhile HPV-2 and HPV-4 preferentially infect squamous epithelium of fingers, producing verrucae vulgaris. Other types, as 6 and 11, infect almost exclusivelly stratified epithelia of oral and anogenital mucosa, producing laryngeal papillomatosis, and condyloma acuminata respectively. Over 100 types of isolated HPV may be divided into three large groups. Mucocutaneous group contains types that infect the skin and the oral epithelium. Another group includes viruses isolated from patients with verruciform epidermodysplasia, a rare immune genetical disorder, in which the patients frequently develop cutaneous HPV-associated lesions that may progress to invasive carcinomas, when sun-exposed. The third group, that contains more than 40 types of HPV, infects the anogenital tract. These associations are not absolute, certain cutaneous HPV, as type 2, may infect mucosal epithelia and HPV-16, considered as a genital type of HPV, was found in association with squamous carcinoma of conjunctiva and of subungual region (7).

HPV groups with oncogenic risk

Based on their association with specific lesional types, the most spread anogenital HPVs were divided in three groups with oncogenic risk. The group with low oncogenic risk includes HPV 6 and 11. These viruses are considered to be of low risk because are often associated with condyloma acuminata from anogenital tract, ocasional associated with LSIL, rare associated with HSIL and almost never associated with cervical invasive squamous carcinoma. Types 42, 43, 44 of HPV are included in low risk oncogenic group because they have the same distribution as HPV 6 and 11. HPV types of high oncogenic risk are 16, 18, 45, 56, and 58; these are considered of high risk because they are most often associated with invasive carcinomas of anogenital tract (8). Beside these, there are other types of HPVs which also have common aspects with high oncogenic risk viruses, because they can be find in association with invasive cervical cancers, although seldom comparative with typical viruses from the same category. These viruses include types 31, 33, 35, 39, 51, 52, 59, and 68, beeing sometimes named viruses with intermediar oncogenic risk. Recent studies indicate the fact that anogenital infections with these viruses present relativ similar risks for HSIL or cancer as the infections with high risk typical viruses. In clinical trial ASCUS LSIL of National Institute of Cancer, it was observed that 83% of women with LSIL from Papanicolau test had an high risk HPV, using DNA HPV Hybrid Capture II, which detected HPV 16, 18, 31, 33, 35, 39, 45, 51, 51, 56, 58, and 66. These studies indicate evidently that LSILs can be associated with low risk and high risk oncogenic HPVs. Unlike this, it exists a more close correlation between histology and associated HPV type, in HSIL lesions. In PCR (polymerase chain reaction) study, Lungu et al observed that only 70% of HSIL contained more than one type of HPV and 88% were associated with HPV 16, 18 or 33 (9).

Particularities in genomic organization of HPV

Genomic organization of variable types of HPV seems to be similar. Viral genoma may be divided into three regions: a circumscribed region (upstream regulatory region, URR, long central region, LCR), early region (E) and late region (L). URR or LCR is a non-codifying of the viral genome important in regulation of viral replication and sequences transcription downstream from early region. Both early and late regions contain a serial structures (open reading frames, ORFs), that represents genomic regions that lack stopping codons, so they are potentially translated into proteins. Early region (E) is early transcribed during the cellular cycle (herewith the source of its name) and codifies predominantly important proteins in viral replication, oppositely to the late region that codifies viral structural proteins lately produced during the cellular viral cycle. URR is a highly complex regulation region, of approximately 400 pb/kb that contains a complex system of superimposed situses for a number of different transcriptional

activators and inhibitors. Six different ORFs, named E1, E2, E4, E5, E6 and E7 were identified in the early region of HPV. E1 codifies two distinct proteins that play a role in extra-chromosomal viral replication. E1 HPV protein has ATP-ase activity. E2 codifies two proteins for DNA binding that regulates transcription. One of the two proteins, E2, has an important regulatory activity. E2 proteins are also important in early region ORFs expression regulation (10). Two of the major key proteins regulated by E2 are E6 and E7; E6 and E7 ORFs codify major transforming genes of HPV. E2 expression inhibits E6/E7 promoter, resulting in E6 and E7 intracellular reduction. E2 overexpression inside cells results in apoptosis (11, 12). HPV E5 ORF codifies a protein with weak transforming activity. E4 ORF codifies serial proteins important in viral maturation and replication. E4 protein function is not completely understood. E4 has several structural protein characteristics, being similar to proteic products of the late region. As the capsid proteins of the late region, L1 and L2, E4 is lately expressed during the viral cellular cycle, in a moment when viral production occurs. E4 function may be that of disorganize the corneum cell envelope, permitting the eliberation of viral particles from the cell. E4 may be also associated with the network of intermediary filaments of keratinocytes, sometimes producing the disorganization of this network. Late HPV region is localized upstream early region and contains two ORFs, named L1 and L2, that codifies viral capsid proteins. L1 protein is the main capsid protein and is highly conserved in all papilloma viruses in all species. L2 proteins is a minor capsid protein, with a higher variability among viral types. Transcription from L1 and L2 occurs as a late event during the viral cellular cycle, in a certain moment when the infectant virus is produced. Transcription from the late region is regulated by regulating transcriptional cellular derived factors produced only by differentiated cells of the superficial and intermediary layers of the squamous epithelium. Consequently, high quantities of codified capsid proteins L1 and L2 may be detected in condyloma acuminata and in LSIL, but these proteins are present only in reduced quantities in HSIL and in cervical cancers. Capsid proteins L1 and L2 produced in vitro, in cellular cultures are capable of association and production of viral type particles (viral-like particles, VLPs) similar to native virions, but without viral genome. In present, VLPs are tested for prophylactic HPV vaccines (13).

Lyfe cycle of HPV - progression

Although the HPV lyfe cycle is not completely characterized, the rough outlines of the process are known. It is considered that initial site of infection is represented either by basal cells or primitve basal-like cells of immature squamous epithelium, which may result for the presence of specific receptors for HPV on the basal cells. One potential receptor that has been localized to the basal cells of the stratified squamous epithelium are integrin complexes containing alpha-6 integrin complexed with either beta-1 or beta-2 integrins (14). Once HPV enters into the basal cells, it can exist within the cells in two distinct biologic states. One is a nonproductive infection in which HPV DNA continues to reside in the basal cells but infectious virions are not produced. In the literature, nonproductive HPV infections have frequently been referred to as latent infections. In nonproductive latent infections, a small number of copies of the HPV genome usually remain in the nucleus in a free circular form called an episome. Replication of the episomal DNA in latent infections is tightly coupled to the replication of the epithelial cells and only occurs in concert with replication of the host cell chromosomal DNA. Because complete viral particles are presumably not produced in latent infections, the characteristic cytopathic effects of a HPV infection are not present and HPV can only be identified using molecular methods. Latently infected epithelium displays no morphologic abnormality. The other form of HPV infection is a productive viral infection. In productive viral infections, viral DNA

replication occurs iindependently of host chromosomal DNA synthesis. This independent viral DNA replication produces large amounts of viral DNA and results in infectious virions. Viral DNA replication takes place predominanlty in the intermediate and superficial cell layers of the stratified squamous epithelium. As the virally infected epithelial cells mature and move toward the epithelial surface, cell-derived, differentiation-specific transcriptional factors produced by the epithelium stimulate the production of viral capsid proteins. This process allows large amounts of intact virions to be formed and produces the characteristic cytopathic effects of HPV that can be detected cytologically and histologically. These cytopathic effects include acanthosis, cytoplasmic vacuolization, koilocytosis, multinucleation, and nuclear atypia (15).

Epidemiologic correlations of HPV genital infection

Epidemiologic studies show that the prevalence of anogenital HPV infections in virgins is extremely low but that large numbers of young women come in contact with anogenital HPVs once thay initiate sexual intercourse. In a prospective study of 100 virgins from Denmark, all were found at enrollment to be HPV DNA negative and seronegative for antibodies against HPV 16 (16). In general, anogenital HPV infections tend to be transient and of relatively short duration in both young and older women. Because most HPV infected women spontaneously resolve their infections, the prevalence of HPV infections decreases with increasing age. Therefore, the natural history of HPV infections is that most sexually active young women are exposed to the virus at some point after initiating sexually activity. Most of these women develop transient HPV infections that are of relatively short duration, and eventually most of these HPV infected women will become HPV DNA negative. Only a small proportion of women exposed to HPV become persistently infected and continue to have detectable levels of HPV DNA in the genital epithelium. These women are from high risk HPV infected category and, through their persistent infection, present the risk of developing invasive cervical carcinoma. It is considered that an important role in the evolution of an HPV infection have the immunologic factors and viral type. The role of immunologic factors is demonstrated by the finding that persistance of HPV infections is much more common in women HPV infected than those noninfected and the persistance rate in this category of population rise with increasing of immunosuppression levels.

The molecular mechanisms involved in malignant conversion determined by HPV

Molecular researches which used cellular and tissue cultures gave an understanding of mechanism through which HPV transform the cervical epithelium. High risk HPV types, as HPV 16 and 18, produce three proteins with growth-stimulating and transforming properties, E5, E6, and E7. E5 is not essential for transformation as the E5 region is frequently deleted in cervical carcinoma cells (17). The E6 and E7 ORFs represent the principal transforming genes of HPV. The E6 and E7 genes complement each other and are only weakly active when introduced alone. In addition to having in vitro trasnforming activity, both E6 and E7 are almost always actively transcribed in cervical cancer, suggesting that the over- or unregulated expression of these genes is required for the maintenance of the transformed malignant phenotype. Oncoprotein E7 accounts for the major transforming and immortalizing activity in high risk types of HPV. E7 is a small zinc-binding protein composed of approximately 100 amino acids that is phosphorylated in the native state and lacks enzymatic activity. E7 contains also a binding site for retinoblastoma gene (Rb) (18). Rb is involved in regulation of cell proliferation, suffering various phophorilation degrees during cell cycle. PRb inhibits also the transcription of an inhibitory gene of cyclin-dependent kinase p16 (INK 4A), with a role in cell cycle proliferation. Through blocking of pRb function is produce overexpression of p16 (INK 4A) in cells. It was recently suggested that the increased expression of p16 can represent a marker for SIL lesions associated with high risk HPV

types. E7 proteins interrupt the control of cell proliferation, inactivating the kinase inhibitors cyclin-dependent p21 CIP1 and p27 KIP1. E6 oncoprotein of HPV has an relatively weak independent activity of transformation and immortalization, comparative with E7. E6 protein is a small protein with two zinc-binding domains, with a length of approximately 150 amino acids which exerts the effects through interactions with regulatory proteins of the cell cycle and which lacks the endogenous enzymatic activity.

The role of p53 in HPV lesions

All these proteins have the capacity of binding a variety of important regulatory proteins, as p53. p53 is an important cell regulation gene which actions as an transcriptional activator and has the characteristics of a tumoral suppresor gene. The lack of expression of wild type of p53 is associated with the development of malignancy. Moreover, p53 has the characteristics of an oncogene, because the mutation formes of the gene can action as a dominant transforming gene. In noninfected cells, the levels of p53 increase as a response to DNA or cellular destruction or to aberrant signals of cell proliferation. In virus infected cells, HPV levels are low because the p53 binding to E6 proteins of high risk HPV types produces rapid proteolitic desintegration of p53 bound, through ubiquitin-dependent pathway; this reduces the p53 amount from the cell and causes a lost of repairing mechanism of p53. Mutational analysis of E6 and E7 indicates the importance of their binding to Rb and p53, in transformation and immortalization of these oncoproteins. E7 proteins of high risk HPV subtypes, as HPV 16, eager bounds to Rb, while low risk HPB types, as HPV6 bounds Rb with a much low affinity. The same aspect is met at bounding E6 proteins with p53.

EGFR role in HPV lesions

EGFR (epidermal growth factor receptor), angiogenesis factor present in different tumors, seems to have, together with cerbB2 and c-myc, an important role in prognostic of advanced cervical cancer, but their participation in early stages or in formation of preneoplastic lesions is still noncleared up. Human EGFR gene is localized on chromosome 7 and codifies a surface transmembranar glycoprotein which binds EGF, TGFalpha, amphiregulin, and HBEGF (heparin-binding growth factor). In EGFR activation may be envolved HPV-E5 oncogene and this may be produced without concomitent growth of receptors's number (19, 20). HPV-E6 oncogene may establish afterwards the increase of EGFR mARN level and the stabilization of the protein, thus in the cells will increase the signal transduction. HPV-E5 establishes an acceleration of Her-2/neu, cerbB2gene protein activation. The association of EGFR expression with the tumoral prognostic in cervical cancer is still noncleared up and controversed, being mentioned years before, abandoned and recently resumpted.

COX2 role in HPV lesions

Cyclooxygenase 2 (COX2) regulates the prostaglandins production and it seems to have a role in appearance and progression of different malignant tumors, but the action mechanism is still noncleared up. It was established that the expression of isoform COX2 is induced by the cells involved in inflammatory process and also by certain tumoral cells (21). It seems that the metabolic rests produced by the COX2 action against arahidonic acid are involved in some carcinogenesis mechanisms. Some cellular elements from chronic inflammatory processes, together with stromal cells may be involved in neoplastic transformation of proliferant stem cells and in the process of tumor invasion. A big number of tumors, among which is cervical carcinoma, can express COX2 in association with glutation-S-transferase isoenzymes and can be considered as possible molecular targets in antitumoral therapy.

Evolutive aspects of HPV lesions

There is a median 10 years latency between initial HPV infection and cancer development, in vivo.

Supplementary, only few patients high oncogenic risk-HPV exposed consequently develop cancer. These observations suggest that the events or additional factors, that may include cromosomial instability induction, with aneuploidy development, are probable important for cervical cancer development. As we previously mentioned, the infection of epithelial cells with high-risk HPV represents an essential request for cervical cancer development. Numerous researches had demonstrated that HPV DNA presence is necessary for the development and persistency of cervical neoplasia and DNA disappearance anticipates the regression of neoplastic cells, even in an advanced lesion (HSIL). Even though infectious lesions may be detected on cervical smears several years before the onset of invasive squamous carcinoma, there is no morphologic criterion that may predict if a LSIL will disappear or will progress toward cancer. High risk DNA HPV detection in such lesions select women with high risk for cervical cancer development, but is not a method that may be independently used in risk evaluation: up to 59% of LSILs have positive DNA, a lot of them registering spontaneous regression, during follow-up, with viral genome dissapearance. Consequently, high risk HPV DNA persistence and morphologic progression of SIL have to be considered in association with in order to select patients which require treatment. In CIN 1/2 equivalent lesions, viral replication is evident in superficial layers of the squamous epithelium, leading to L1 major capsid antigen expression. This allows the immunohistochemical detection of infected cell nuclei. Anyway, in a certain proportion, this method do not detects L1 capsid in LSIL lesions. No marker about morphologically identical SILs, but with opposite prognosis significance, was yet identified. Regarding this matter, viral charge, viral integration or markers of viral and cellular genetical interactions may be significant.

L1 capsid protein expression

L1 capsid protein is expressed in the active phase of HPV infection and is necessary in viral cellular cycle completion. Consequently, viral protein detection, by immunohistochemical reaction is an evidence of active HPV infection in examined tissue (22). L1 viral capsid protein is considered a major target of the cellular immune response (23). LSIL and moderate SIL without immunohistochemically detected L1 are correlated, in more than 80% of cases, with dysplasia progression. Moser and co. certify these aspects, evidentiating that minor and moderate lesions without L1 capsid protein expression are significantly more exposed to a progression in comparison to L1 positive cases (24). Most probably, the lack of HPV antigen is determined by a weak protein synthesis, under immunohistochemical test minimum level. As L1 represents the major target of the immune cellular response 25), its deficient translation may result in an inefficient epuration of the infected cells, promoting viral DNA integration in host cellular genome and the transformation of immature epithelial cells. The observation that the decrease of the HPV16 capsid positivity in cervical cancer patients serum is an indicator of a poor prognosis sustains the importance of a specific humoral response. Immunohistochemical detection of L1 capsid, on conventional Papanicolau smears, may consequently indicate the defence status locally induced on HPV infection and may offer prognosis information in LSIL lesions.

The role of antiHPV vaccination

Recently there were developed vaccines to prevent the HPV infection and respectivelly the development of an cancer (26, 27, 28). The vaccines contain adjuvants HPV VLPs produced through expression of L1 viral capsid protein in eukariotic systems. These vaccines induce

increased levels of neutralising viral antibodies pointed toward conformational determinants of capsid protein (29, 30).

CONCLUSIONS

Our opinion is that in case of HPV infection, the immunity system of the host reacts either through anti L1 capsid antibodies production (humoral response) and through anticapsid cytotoxic cells (cellular response), the infected epithelial cell expressing the major protein capsid. Consecutive, a marked expression of the capsid protein is correlated with a increasing of the immune response, the loss of expression suggesting the decreasing of the immunity. These connexions can become important for the cervical tumoral pathology domain, the impact upon the specific area resulting from the developing of new algorythms of LSIL, HSIL, and ASCUS management, algorythms which have as main pillar HPV vaccination not only in the absence of HPV infection, but also in the presence of an latent infection.

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