THE ETHIOLOGICAL ROLE OF HPV IN THE CERVICAL CARCINOGENESIS

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Rezumat: *Cunoştinţele privind cancerul cervical şi patogenia sa au înregistrat o dezvoltare remarcabilă. Cercetările făcute cu metode moleculare pentru detectarea şi analiza HPV (Papilomavirusul uman) au ameliorat înţelegerea în măsura în care au fost formulate noi criterii morfologice. Coexistenţa leziune cervicală - HPV este indiscutabilă. Scopul lucrării este de a prezenta rolul etiologic al HPV în carcinogeneza cervicală, precum şi rolul oncoproteinelor HPV în instabilitatea genomică.*

Keywords: *HPV carcinogenesis, cervical cancer*

Abstract: *The knowledge of the cervical cancer and its pathogenesis has known a remarkable development. Research carried out by molecular methods for detecting and REVIEW HPV (human papillomavirus) improved understanding to the extent to which new morphologic criteria were formulated. The coexistence of the cervical lesions and HPV is indisputable. The purpose of this paper is to present the etiological role of HPV in cervical carcinogenesis and genomic instability in HPV oncoproteins role.*

SCIENTIFICALLY ARTICLE OF BIBLIOGRAPHICAL SYNTHESIS

In the present degree of researches , one may consider that the process of cancerisation of the cervix is initiated at a young age, as a consequence of the interaction between a carcinogenetic agent (probably viral), introduced with the first sexual rapports in the cervical epithelium incompletely maturated, unprepared for a permanent sexual life.

After a long period of latency (15-20 years),on the background of existence of a reversible cervical lesion ,it is produced the proper cancerization (in which the viral organism may have a carcinogenetic role next to other eventual factors and/or hormonal influences), with the initial appearance of the intraepithelial neoplasia, then of the invasive one.

The first genital HPV type isolated was HPV 6 (1980). The isolation of the genital HPV directly from the biopsies of the cervical cancers and the identification of the cervical lines derivated from the tumoral tissue that contained HPV genome permitted the expanding of the research area. DNA - HPV specifically was descovered in more than 96% of the precancerous cervical lesions and in 92% of the prelevated biopsies in cases with cervical cancer . There have been described more than 70 HPV types. The association between the infection with HPV and the etiology of the cervicale neoplasia is framed in the epidemiological criterion regarding the causality: numerical loss, consistence and specificity of the associations, temporal relation and biological plausibility.

The basal cells are the first infected cells with HPV. It is supposed that the microtraumas produced through the sexual act favors the acces to those cells. After the core is penetrated by the viral genom the , E genes are activated and the viral DNA replication is realised, the transcriptional control and the cellular transformation . The episoms are the result of the replication in the initial phases of the infection and realises a constant number of viral replicas. Those may maintain in a latent status in the basal cells or may induce the active infection of the parabasal cells and the stimulating of the functions of the genes E and L in the cells that are about to differentiate. Those effects may become clinically manifest in intervals of 1, 5 till 8 months since the installation of the infection.

The genital infections with HPV are classified in clinical, subclinical and latent and are diferentiated by the precancerous lesions and invasive as neoplasia associated to HPV. The prevalence of the latent infections and subclinical ones depends on many factors: the sensitivity of the detection method of the HPV, the presence of the associated neoplasia HPV, age and the characteristics of the sexual life, the number of tests realised at each subject. It is considered that HPV infects reserve cells from the level of the transition zone, cells with a potential of squamous differentiation, glandular or neuroendocrine. The classification of the different types of HPV is based on the sequences of the genic nucleotides and is better correlated with their cellular tropism and with the oncogenic.potential The genital HPV type are classified in two groups in accordance with the oncogene risk: the group with a low risk of the progression of the lesion to cancer and the group with a moderated or high risk. The first group is defined by the fact that ,those viruses are almost never decelated in the invasive cancers (table no. 1).

Table no. 1. The classification of the HPV in accordance with the oncogenous risk (după L. L. Villa, 1997)

Risk category	Type
Low risk	6, 11, 26, 42, 44, 54, 70, 73
High risk	16, 18, 31, 33, 35, 39, 45, 51, 55, 56, 58, 59, 66, 68

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Molecular genetics of the cervical cancer

In the process of the carcinogenesis, in general,there are incriminated the following three categories of genetic modifications:

- The loss of a distinct chromosomial regions;
- The activation of the oncogenes;
- The inactivation of the suppressor genes.

With approximativelly three decades ago it arrived to the conclusion that each lesion (CIN or invasive) has characteristics regarding the number of the chromosomes (often with the presence of triploidias with tetraploidias) and of the presence of the abnormal chromosomes („markers"). The analyses of the karyotype showed that several chromosomes present more frequently structural modifications. The modifications of the 1st chromosomes (the loss of the short arm , the duplication of the long arm, deletions, translocations, isochromosomes) were observated in 60% of the cases.

It has been observed also that,in the cases that presented those structural modifications,the prognosis at 5 years was more reserved. The deletions of the 3rd chromosomes are quoted in 26% of the cervical cancer. At the level of the 5 chromosomes was indentified the isochromosome of the short arm and the 11 chromosome may present deletions and translocations.

The structural modification (translocations, isochromosome) of the 17 chromosomes are described in over 40% of the cervical carcinoma. It is known that the suppressor gene p53, localized on this chromosome, may be inactivated by the HPV oncoproteins. The p53 mutations were observed concomitently with the allelic loss. The loss of the heterozigot on the short arm of the 17th chromosome is relativelly frequent in the cervical cancer. In some cancers it was detected also the alteration of p16.

It is considered that, excepting the simple dysplasia, every lesion is clonal and is characterized through a particular aneuploid complement, frequently poliploidy. The clinical symptoms, inclusive the neoplasic ones, are developed by a reduced percentage of women infected with HPV – great risk. This may help us to conclude that, the viral infection constitutes just a step toward the cervical carcinogenesis, for the development of the cancer being necessary other cellular events. The oncogene activation, as a consequence of the viral integration, may be in a relationship with the chromosomial anomalies.

The inactivation of the oncogenes may represent a mechanism of reactivation of the apoptosis ways and of the cellular supervision with effects in the elimination of the cancerous cells. The main functions held by p53 „the guardian of the human genome", consist in the understanding of the affection of the cellular DNA and the prevention of the division of the compromised cells . The inactivation or mutation p53 permits the replication of these cells, fact that determines the growing of the genome instability. The cells that express the oncoprotein E6 (HPV with a higher risk) are not blocked in the G1/S phases after the affectation of the DNA.

How it was mentioned before, in the multi-phase process of the carcinogenesis are recognised valable genetic alterations for many tumoral types: the super-activation of the oncogenes and the inactivation of the suppressor genes. The HPV sequences are integrated in the genome of the cancerous cells and they may be involved in the oncogene activation. Some proto-oncogenes are involved in the malign transformation of the cervix.

It is well-known the fact that the proto-oncogenes are part of the genetic cellular patrimony and encode proteins with an important role in the growth control. The defects of the regulations of these genes or structural alterations may transform them in oncogene characteristic to the human cancer. In CIS were observed modifications of the 1st and 11th chromosomes. These modifications are more evident in the invasive squamous carcinoma. The greatest interest was generated by the 11th chromosome, currently affected in a variety of solid neoplasias.

The molecular genetic studies are suspecting the presence of a suppressor gene on this chromosome, gene that could be important in the initiation and progression of the cervical cancer. Introducing a normal 11th chromosome in a malign cell derived from a cervical squamous carcinoma (that has the 11th chromosome abnormally) may affect some characteristics of the malign phenotype.

The role of the HPV oncoproteins in the genomic instabillity

HPV plays an important etiologic role in the development of the cervical neoplasia. 99,7% of the cervical neoplasia are caused by types of HPV with high risk, HPV 16 and 18 being the most frequent met in the invasive cervical neoplasia in the majority of the population. Clinical and experimental studies suggest that the infection with HPV 18 is associated with more aggresive forms of cervical neoplasia compared to HPV 16. HPV 18 is the most often met in the cervical adenocarcinomas.

The life's cycle of the HPV is closely linked to the way of differentiation of the infected epithelium. HPV infects initially the basal layer of the epithelium. The nature of the HPV receptors is still unclear, the integrin 24 B6 having an important role. Similarly, the process that mediates the integration of the virus, decapsidation and the nuclear import of the viral genome is not completelly known. The frequency of the HPV integration grows with the degree of the disease's severity, correlating with the evolution of the cervical neoplasia. The epigenetic modifications in the HPV genome may alert the gene expression (de ex. Phenotype), but without any modifications in the DNA sequence (for example Genotype). The exemples are the hypermethylation or the hypomethylation of the viral oncogenes and their potential implications regarding the suppression and/or the activation of the viral oncogenic expression.

There is a positive association between the identification of the HPV antibodies (humoral imunity) and the risk of apparition of the cervical neoplasia. Although it is considered that those antibodies may prevent effectivelly the infection, it seems that they may not have effect in the regression of a HPV infection installated or in the cervical lesions. In contrast with the anti-bodies, the answer of the T cells (cellular imunity) of the HPV represents an important effector mechanism in the eradication of the present infection. So, the answer of the T cells may help to the protection against the progression of the infectious process or of the incipient lesions.

Viral DNA is maintained in the nucleus of the infected host cells, that is differentiated further and arrive at the surface of the epithelium. In the epithelian differentiated cells,the virus is replicated in a large number of copies.

The E4 HPV protein is associated with intermediate keratine filaments, affecting the mechanical stability of the keratine network and facilitating this way, the release of the viral particles.

The E1 and E2 proteins of the HPV have an important role in the process of viral genome replication. The E2 protein is a factor of transcription linked to the DNA that interacts with the fragment from the LCR portion of the HPV virus (the control region of the HPV that contains a variety of *cis* elements, that

adjusts the replication and viral transcription).

The E2 proteins with high risk of the HPV have the capacity to act as transcriptional activators, but they may function also as a transcriptional repressor of the viral gene expression at the level of the keratinocytes. The E2 HPV proteins have an essential role in the transcriptional adjustement and replication of the viral DNA.

Moreover, for the modulation of the viral transcription, the E2 HPV proteins are associated with the E1 helix of the viral DNA. This interaction is necessary for the recognising and viral gene replication. So,it is important in the viral gene segregation during the cellular division through the fixation of the viral genomes at the level of the mitotic chromosomes, association mediated through the interaction with the Brad 44 protein. The concept that the, loss of the repressive function of E2 may be critical for the malignant progression is sustained by experiments showing that, the re-expression of the E2 in the cellular lines of cervical neoplasia determines the suppression of their development. Those experiments suggest clearly that continuous expression of the E6/E7 in the cervical neoplasia is necessary to maintain the transformed phenotype.

One of the key events of the carcinogenesis indused by HPV is the integration of the HPV genome in the host's chromosomes. The integration pursues a specific model, that respects the HPV genome. The expressionof the viral genes E6 and E7 is maintained constant, while other proteins of the viral DNA are wiped out or their expression is troubled. The loss of the expression transcriptional repressor E2 HPV is significant because it may determine the disorder of the expression of E6 and E7 HPV.

The integration of the viral genome in the chromosomes of the host cells determines the loss of the E5 expressing. The E5 protein is associated with the intracellular membranes and determins the transformation of the cells through the activation of the tyrosine kinase receptor.The E5 HPV proteins have similar activities, the disorder of the E5 expression may affect the life cycle of all the high risk types of HPV. The fact that the expression of E5 isn't detected frequently in the cervical neoplasia after the integration of the viral genome proves that, E5 isn't necessary for the maintaining of the transformed phenotype.

The types of HPV „high risk" encodes two oncoproteins E6 and E7.The expression of the E6 and E7 HPV genes isn't necessary only for the induction of the pre-malignant transformations, but contributes directly to the maligne progression through the submination of the genomic stability. The transition from normal to dysplasia or invasive carcinoma is realised through the uncontrolled expression of the E6 and E7 at the level of the proliferative epithelial basal and parabasal cells. The function of those oncoproteins is of stimulating the cells proliferation through the interferation with the regulation proteins function in the cells, including the products of the suppressor tumoral genes p53 and pRb. E6 is constituted from approximativelly 150 aminoacids and has the role of inactivating p53, preventing the cells apoptosis mediated through p53. Moreover, E6 HPV may activate the transcription of the TERT (the catalytic subunit of the human telomerase) . The E6 HPV protein may form a complex with the p53 protein. E6 is not associated directly with p53, but constitutes a complex with the R 6-AP protein, determining a rapid proteazomal degradation of the p53. Each DNA replication determines the erosion of the chromosomial telomeric region. The telomeric shortening represents a cellular autonomous mechanism that restrictions the proliferative capacity of the normal somatic cells. Ectopic expression of the catalytic subunit, hTERT, in the human primary cells determines the prolongation of the life cycle.The majority of the tumoral cells telomeraso - pozitiv, suggesting an aberrant telomerase activity, that may be critical for the human tumorigenesis. The E6 proteins induce the expression of the hTERT and interaction directly with c-myc. The alterations of the c-myc gene may be associated with the integration of sequences of HPV 16 in genital tumors, including the activation of the proto-oncogenes, a mechanism of insertional mutagenesis and/or gene amplification.

The E7 HPV protein has a low molecular weight and consists from approximativelly 100 amino-acids. This interacts with pRB, p107 and p130. Alongside the regulation and degradation of pRB, E7 has also other important cellular targets. E7 HPV may not defer to the inhibition activity of the growth determined by p21 KIP1 and p27 KIP1, inhibitors dependent on cycline. As long as this proteins are critical factors of regulation of the cellular cycle during the keratinocyte differentiation, their inhibition through E7 may contribute at the maintaining a competent modality of replication in the host epithelial differentiated cells.

E7 forms a complex with the members of the retinoblastoma family (pRb) and inactivates their functions of growth suppression. Moreover, it has been showed that the E6 and E7 proteins link a variety of other cellular proteins, contributing to the cellular transformation. E7 is linked to the pRb determining hyperproliferation and induces a process of abnormal duplication of the centrosome that is independent of the pRb inactivation or of a member in the gene pRb family.

There is the evidence of a growing stability of the ADNm E6/E7 HPV 16 after the integration, specific alterations of the gene expression of the host cells are detected after the integration of the HPV genome. Those experiments suggest clearly that, the continous expression of E6/E7 in cervical neoplasia is necessary to the maintenance of the transformed phenotype.

The HPV infection itself is inssufficient to induce cervical cancer, but other factors, such as the steroid hormones seem to have a role in establishing and/or progressing of this disease. E2 protein has a role in the viral replication and modulation of the expression of the viral gene, whereas the E7 HPV 16 protein is implicated in the cellular transformation. It was observed that both proteins E2 and E7 may induce cellular apoptotic death in the cellular lines transformed non-HPV and the ones HPV transformed. The steroids hormons estrogen and progesterone may increase the levels of the apoptosis induced by E2 and E7.

The ras mutations, that are produced in a subset of cervical cancers, may contribute to pathogenesis. So, it was observed that the protein encoded by E7 of the HPV 16 whereas the affectation of the controll of the host cells growth, through the linkage of the pRb protein, contributes to the maintaining of the malignant phenotype, through the modulation of the CMH-1 expression such the case of c-myc oncogene. This formed complex, pRb-E7, may determine the hyperexpression of the c-myc. C-myc, along with the controll of the growing and proliferations of the cells, contributes at the maintaining of the malignant phenotype through the modulation of the CMH-1 expression.

The E6 and E7 onogenes may cooperate with the oncogenes ras in the human epithelial transformed cells, and the Ha-ras oncogene induces transcriptional activity of the viral promoters, determining a growth of 2-3 times of the E6/E7 transcripts. So, Ha-ras regulates the expression of the HPV oncogene through the modulation of the AP-1 activity and suggests that the grown levels of the E6 and E7, resulted from the viral transcription activated in the presence of the ras oncogenes, may explain partly the cooperation between those

cellular and viral oncogenes in the transformation of the human cells.

The biomarker p16 is very useful in the differentiation of NIC of the reactive lesions. The expression of p16 is realised through a negative feed-back dependent of pRb, the continous of the pRb by E7 HPV determines a growing concentration in p16. So, the growing concentration in p16 may explain the dysplasia induced by HPV.

Ki67 is one of the most predictable biomarkers of the neoplasic cervical proliferation. Its immunoquantitative determination represents an adjuvant diagnosis in the NIC. The combination between the stratification index (IS) – that shows how profoundly are placed the positive Ki67 nucleus in the epithelium (as bigger the SI, as serious the degree of NIC) and the number of nucleus Ki67/100 µm from the basal membrane, being the best discriminative set that differentiate the 3 types of NIC.The presence Ki67 in the cervix biopsy indicates the progression of the incipient lesions in NIC3.

CONCLUSIONS

- The risk of HPV infection on the life period among the sexual active persons is at least of 50%;
- Although the majority of the infections are eliminated with the help of the personal imunity, the infected persons aren't aware by the presence of the HPV and may propagate the virus;
- In the conditions in which the personal immunitary system can not eliminate the infection, the persistence of the viral oncogene stems in the cervical mucosae may determine the apparition of the pre-cancerous lesions and then of the cervical cancer;
- The cervical cancer is caused by the oncogenic types of HPV, the types 16 and 18 being responsable in 70-99,7% of the cases.

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