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REVIEW

Virus-Associated Human Tumors: Cervical Carcinomas and Papilloma Viruses

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Abstract—The latest experimental data on the role of viruses in the origin of human tumors are discussed. This group of viruses consists of T-cell leukemia virus type 1 (HTLV 1), herpes viruses (HHV 8 and Epstein–Barr virus), hepatitis B virus, and human papilloma viruses. The most typical feature of this group of viruses is a very long latent period from the initial infection to the development of the disease that varies between 10 and 40 years. The mechanism of malignant cell conversion is specific for each viral type but is mainly associated with a disruption of functions of cellular genes participating in the control of cell division and proliferation. It can be a direct inactivation of tumor suppressor genes by their interaction with viral gene products (papilloma viruses), or a trans-activation of cellular genes modulating cell proliferation by viral gene products (hepatitis B virus and HTLV 1). Viruses play an initiative role and additional genetic changes in the genome of infected cells are necessary for complete expression of the oncogenic potential of the viral genes. Only these cells will give rise to a monoclonal cell population with uncontrolled proliferation. New approaches for the creation of vaccines against cancers associated with hepatitis B virus and papilloma viruses (hepatocellular carcinomas and cervical tumors, respectively) are in progress. These vaccines have been found to be effective in prevention of the disease in the experimental models and are now beginning to be used for human vaccination.

Key words: tumors, cervical cancer, transformation, mechanisms, papilloma viruses, transforming genes, oncogenes, tumor suppressor genes

It is well known that the life cycle of a cell is controlled by its own genes and is strictly regulated. The majority of cells in an organism, as well as cells cultured *in vitro*, have a limited life span and their death is programmed in advance. A malignant cell differs from a normal one mainly by its independence from this control. Instead of passing to apoptosis, this cell continues in uncontrolled growth leading to the formation of a tumor. Thus the malignant cell is switched over from one genetic program (apoptosis) to a principally different one that leads to immortalization. Clear evidence for the participation of several cell genes in this process, especially oncogenes, tumor suppressor genes, and genes regulating the cell cycle, has been provided. However, the molecular mechanisms of the "oncogenic" program induction in the cell remain unknown for the majority of tumors.

The tumors induced by viruses partly resolve this problem since they allow identification with a relatively high probability a viral gene stimulating the process of uncontrolled cell proliferation and following this signal pathway in the cell.

Therefore, the role of viruses in carcinogenesis seems to be one of the most interesting problems not only for oncology, but also for biology in general. The role of viruses in the origin of tumors has been established for various tumors of mammals and birds. They are able to induce cell transformation *in vitro* and tumors *in vivo*. The genes whose products directly control the transformed phenotype (oncogenes) of the infected cells or the genes whose products induce the synthesis of other genes involved in the control of cell proliferation have been identified within the viruses of this group. Both viral types represent very good models for the analysis of the changes in the biological behavior of the cells as a result of the interaction between various gene types.

Although, as mentioned above, viruses with oncogenic potential have been found in many animals and birds, humans have been an exception for a rather long period of time, since numerous attempts to isolate a virus from various human tumors have failed. The situation changed in the beginning of 1980s when a few DNA- and RNA-containing viruses thought to be related to the formation of tumors according to several criteria were isolated [1]. Today several viruses that are responsible for the development of approximately 15% of all human

tumors have been characterized. These viruses can be divided into two groups. The first group of viruses with direct action consists of papilloma viruses; these are associated with cervical cancer and contain transforming genes. Two other types of DNA-containing viruses are hepatitis B, associated with hepatocellular carcinoma, and two herpes viruses, Epstein–Barr virus, associated with nasopharyngeal carcinoma tumors and Burkitt lymphomas, and herpes virus type 8, related to the Kaposi sarcoma. The hepatitis B virus is attributed to the group of viruses with indirect action, since its genome does not contain any oncogene and its oncogenic potential is realized through the activation of cellular genes participating in cell proliferation. The structure of both herpes viruses is extremely complicated and all experimental data about the oncogenic potential of distinct regions of their genome seem to be preliminary.

One RNA-containing retrovirus, human T-cell leukemia virus (HTLV 1), was also found to be associated with a relatively rare type of leukemia (adult T-cell leukemia, ATL). This virus has no oncogene as well, but one of its genes encodes for several proteins that are capable of activating various factors, including cytokines stimulating cell proliferation.

A very long latent period (5-30 years and more) seems to be a common and typical feature of all virus-associated human tumors. This fact confirms the proposal that even in tumors of viral origin several additional genetics events are necessary for the complete manifestation of the oncogenic potential. These secondary genetic events are only beginning to be studied and their mechanisms are poorly understood.

Since antiviral vaccines may be effective in the prevention of virus-associated diseases, these vaccines are thought to be effective for the prevention of some virus-associated human tumors.

Thus, the above-mentioned data provide evidence that human oncovirology is a rapidly developing branch of oncology that can give an important practical effect.

It seems practically impossible to discuss all experimental data in the field in one review. Thus, we decided to focus on one model that provides the most impressive results concerning the mechanisms of virus-induced tumor development by viral genes and the creation of anti-viral vaccines as well. This model is cervical cancer and the human papilloma viruses detected in these tumors. About 400,000 women develop this disease each year worldwide. This cancer is one of the most frequent after breast cancer [1].

STRUCTURE OF THE GENOME OF PAPILLOMA VIRUSES

A suggestion made in the early 1970s [2] that human papilloma viruses (HPV) could play a causative role in cervical cancer resulted in the rapid expansion of this branch of virology and oncology. On one hand, it led to the appearance of strong evidence of a causal role of HPV in this type of cancer, that was finally confirmed in the Press Release of the World Health Organization (WHO) of July 3, 1996. On the other hand, it favored rapid progress in the investigation not only of this group of viruses, but of the mechanisms of malignant cellular transformation induced by viruses as well.

In this review we are not going to present all data about the role of HPV in cervical cancer (the frequency of viral DNA detection in tumors, its expression, form of persistence, epidemiological data, see [3]), but we would like to focus on the viral genes playing the key role in the malignant cell transformation.

Today more than 100 HPV types have been identified [3]. These viruses are characterized by the absence of an adequate cellular model for their reproduction. Therefore, almost all HPV types were identified by the isolation of viral DNA from the infected cells followed by its cloning and sequencing. Almost all HPVs can be divided into two large groups, those that are associated with the skin or those with the mucosa. The most typical signs of skin infection are benign papillomas, and precancer lesions and cancer of the cervix for mucosal epithelia [4].

All papilloma viruses have similar structure. They contain DNA of 8000 base pairs, which constitutes of nine open reading frames, two of them (L1 and L2) encoding for the structural viral proteins. Other seven frames are attributed to so-called "early" viral genes and control viral reproduction and the manifestation of the pathogenic potential [5].

Among all isolated HPV only a relatively small number is associated with various malignant tumors. Within "skin" HPV types these are viruses detected in epidermodysplasia verruciformis. The squamous cell carcinomas are formed from papillomas after sun irradiation during this disease [6]. HPV type 5 is most frequent and other HPV types (8, 14, 17, 20) are occasionally detected in malignant lesions [7]. Since there are no cellular models of this disease, the mechanisms of viral gene function remains obscure.

The most important data about the role of HPV in human carcinogenesis were obtained on the HPV types isolated from mucosa, mainly from cervical tumors. The pioneering investigation of the group of Dr. H. zur Hausen [8-11] in the middle of the 1980s provided strong evidence that two HPV types (16 and 18) were detected in cervical cancer, while HPV types 6 and 11 were detected in the majority of benign tumors. Subsequent screening of tumors permitted the detection of some other HPV types in cervical tumors and other malignant lesions of the anogenital region of women and men (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 70), whereas the number of new HPV types isolated from benign papillomas

of this region and from oral papillomas was significantly lower (types 3, 32, 72, 73) [12].

Sensitive methods allowed the isolation of HPV DNA from more than 90% of cervical tumors. This is a strong argument supporting the hypothesis that the presence of viral genetic material is an essential factor for the malignant transformation.

The data presented above allowed all HPV isolated from benign and malignant neoplasias to be divided into two classes: so-called HPV of "low" and "high" risk. Analysis of these HPV classes did not reveal any principal structural differences in the viral genome: HPV of both classes contain two structural genes (L1 and L2) and seven functional genes (E1-E7). In addition an upstream regulatory region (URR) was detected in all HPV. The URR is localized directly upstream of the genes E6 and E7. A significant number of sites able to interact with positive and negative transcriptional factors was identified within the URR (see below). This interaction seems to play a great role since the HPV infection in squamous and mucosal epithelium is persistent and causes accelerated cell proliferation. To develop an effective infection the virus has to penetrate into basal cells, since only these cells are capable of reproduction in epithelium. These cells give rise to all other cells of upper levels, which loose the ability to divide during differentiation and exfoliate from the surface. Replication of viral DNA, expression of late genes, and viral maturation are closely related to the cellular differentiation and take place only in the upper epithelial levels.

The transcription of different HPV genes mainly depends on the cellular transcription factors on the early stages of the infection after the migration of viral DNA to the nucleus. On the latter stages viral factors also participate in the regulation of the expression of early and late viral functions.

HPV infection of the cervix is characterized by viral sequences detected not only in malignant tumors (carcinomas), but in pre-cancer lesions (so-called intraepithelial dysplasia, CIN) of different levels as well. This may indicate that the virus initiates the disease, and the cellular genetic factors play a crucial role in the further malignant progression.

The viral genome can persist in episomal and integrated forms in the infected cells [13]. The early data indicated that the episomal form prevailed on the early stages of the process (CIN), while in carcinomas viral DNA was integrated into the cellular genome [14]. However, new data seem to be contradictory. Probably viral DNA is integrated into the genome in the majority of tumorigenic cell lines obtained from cervical carcinomas, whereas the detection of both types of viral DNA persistence is possible in tumors (which typically have a heterogeneous cell population) [15]. The role of the integration in the maintenance of malignant cell status is poorly understood since there is insufficient information about the RNA types transcribed from integrated and episomal viral genomes.

REGULATION OF THE VIRAL GENE TRANSCRIPTION

The results presented above clearly demonstrate that the maintenance of transformed phenotype is controlled by the functional activity of viral genes. The E6 and E7 genes are considered to be the major transforming genes of the human papilloma viruses. The expression of these genes is modulated by the URR. The length of the URR varies between 800 and 1000 base pairs for different HPV types. This region can be divided into three parts—the 5′-region, central segment, and 3′-region which is located directly upstream from the E6 and E7 genes. The E1, E2, and E5 genes are located downstream from the E6 and E7 genes, the E2 gene overlapping the E4 gene. Presumably the E5 gene is also a transforming gene. The signaling terminal sequence for poly(A) is located at the 3′-end of the early region. RNA transcription starts from a promoter in the 3′-region of the URR, directly upstream from the E6 gene. This promoter is referred to as P97 for HPV 16 and P105 for HPV 18. The promoter consists of a TATA-box and a transcription initiation site that are regulated by different enhancers mapped in the central and 3′-regions of the URR. They bind cellular transcription factors. The 3′-region of the URR also contains two binding sites for viral transcription factor E2, cellular factors Sp1 and YY1, and replication origins with binding sites for E1 viral protein.

The central segment of the URR contains a constitutive enhancer that is most active in epithelial cells and is dependent only on cellular transcription factors [16-19]. Sites for various cellular factors including AP-1, NF1, Oct1, TEF1, TEF2, YY1, and for steroid hormones are identified within this region. All these factors are ubiquitous and can function in different cell types. The 5′-region of the URR contains signals for termination and polyadenylation of late viral transcripts [18].

The factor E2 is the only factor of viral origin among numerous transcription factors capable of interacting with the URR, but its activity is important for the function of the HPV transforming genes.

HPV URR contains four E2-binding sites. Data concerning the effect of E2 on the transcription of E6 and E7 genes are contradictory. On one hand, the products of the E2 gene may function as repressors for HPV type 18 promoters [20], and mutations in the E2 gene increase the level of cell immortalization by genes E6 and E7 [21]. On the other hand, some data indicate that E2 proteins of HPV 16 and HPV 18 activate the URR

promoter [22]. Probably, these differences are caused by synthesis of differently spliced mRNA from this gene leading to the formation of truncated forms of E2 proteins. E2 protein without the N-terminal transactivating domain may interfere with the activating potential of a full-length E2 protein [22]. Thus, the regulation of E6 and E7 gene activity by a product of the E2 viral gene is rather complex, since this protein has two opposite functions, as an activator and as a repressor.

Among numerous cellular factors able to interact with the URR, we will mention only those which were found to be essential for the manifestation of E6 and E7 gene functions. First of all is AP-1 transcription factor. This dimeric protein complex consists of one copy of each member of *jun* and *fos* gene family products. HPV 16 and HPV 18 URR contain three and two AP-1-binding sites, respectively. The AP-1-binding site regulates the URR transcriptional activity by selection of the specific transcripts needed for control of differentiation [23]. Another important regulator of URR, the transcriptional factor YY1, functions as an activator and a repressor of transcription depending on viral type, cell type, and the activity of other transcription factors, especially of AP-1 [24, 25]. Another group of sites is able to bind C/EBP (CCAAT-enhancer binding protein) [26]. The factors of this type may associate not only with corresponding region of DNA in the URR, but bind other cellular factors as well. The major function of this gene group is to regulate cell differentiation and to activate genes of various cytokines. SP1 transcription factor can interact with an element of GC-boxes and its site is located directly downstream of the TATA-box. This factor is essential for the viral genome transcription [27].

Among the transcription factors of the nuclear receptors group, two classes (steroid hormone receptors and thyroid-retinoid receptors) have their receptors within the URR. Glucocorticoid and progesterone receptors belong to the group of steroid hormones receptors [28-30]. The site responsible for glucocorticoid binding (GRE) is localized in the 3′-region between the AP-1- and Sp1-binding sites. Both receptor types activate transcription. A transcription factor specific for keratinocytes, KRF1, can bind to AP-1 for transcriptional activation [31].

The URR also has several binding sites for proteins containing a so-called "POU-domain" that bind a canonical octamer sequence ATGCAAAT (in this case the proteins are referred to as Oct). One of these proteins (Oct1) represses the URR of HPV 18 [32], another one (Epoc1) has an opposite effect [33]. Factors from the NF1 family have several binding sites in the region of a constitutive repressor [34]. These sites have a relatively low affinity and are probably necessary for transcriptional specificity in epithelial cells. Transcription factors TEF1 and TEF2 have four sites within the HPV 16 enhancer and are thought to activate P97 promoter [35].

Thus, the short review on the factors regulating the transcription of HPV transforming genes presented above provides evidence that the transcriptional control is complicated and is regulated by numerous transcription factors having positive and negative effects. They not only bind the viral URR, but interact with each other as well.

TRANSFORMING GENES OF PAPILLOMA VIRUSES

E6 gene. The data mentioned above indicate that the viral genome persists in an integrated form in the majority of malignant tumors. The gap in the viral genome necessary for integration into the cellular genome is located within the E1-E2 frame. This provides evidence for the existence of intact E6 and E7 genes in the transformed cells. The expression of these genes is controlled by the URR. Three types of evidence indicate an important role of these genes in maintaining of transformed cell phenotype: 1) the E6 and E7 genes possess transforming potential *in vitro*; 2) cells transfected with these genes can induce tumors in hairless mice; 3) inhibition of E6/E7 gene expression leads to the reversion of transformed phenotype.

Data concerning the transforming potential of these genes are presented below. The E6 and E7 genes are transcribed from P97 (HPV 16) and P105 (HPV 18) promoters in a form of polycistronic mRNA [36]. Five different mRNA types containing the E6 frame have been identified, but only one of them is a full-length copy. The four other RNA types are truncated as a result of differential splicing within the E6 frame or between the E6 frame and the E1 and E2 frames. The sequences of the latter frames were detected in these spliced RNA. RNA containing a truncated variant of E6 (referred to as E6*) and a complete E7 gene are the most abundant [37, 38]. The role of these RNAs is not yet understood.

The E6 gene product is a protein containing 150 amino acids that does not have any enzymatic activity. E6 proteins are localized in the nucleus, cytoplasm, and membrane fraction [39]. These proteins are relatively highly conservative, and the E6 proteins of HPV from the low-risk group have 80-90% homology; approximately the same level of homology is observed within the high-risk HPV group proteins. Homology of 50-60% was found between E6 of these two groups. E6 proteins of all HPV are characterized by the presence of four zinc-finger motifs [40] typical for DNA-binding proteins; however, all attempts to detect this binding have been unsuccessful.

The study of the biological activity of the HPV E6 gene has demonstrated that it can immortalize human epithelial cells alone and together with the E7 gene as well [41]. In the absence of the E7 gene, the E6 gene was

able to interfere with keratinocyte differentiation induced by serum and Ca^{2+} [42]. In addition, the E6 gene can immortalize human breast epithelium [43] and rodent fibroblasts in cooperation with activated *ras* oncogene [44] and also to initiate tumors in transgenic mice together with the E7 gene [45]. In contrast to E6 of high-risk HPV, E6 of low-risk HPV cannot immortalize or transform the same cells [46].

The p53 gene product is one of the most important targets of E6. It is well known that p53 [47] is a transcription modulator and specifically interacts with DNA, thus transactivating such important genes as $p21^{WAF1}$, whose product is an inhibitor of cyclin-dependent kinases [48], and *bax*, playing a role in apoptosis [49]. The p53 gene is activated during treatment of cells with DNA-damaging agents and hypoxia [50, 51]. A mutated variant of the p53 gene, often isolated from many tumors, is restricted in all these functions, and its localization is limited to the cytoplasm [52].

It was demonstrated earlier that the p53 gene protein could coprecipitate with SV40 large T antigen and the product of adenovirus early E1a gene from cells infected by these viruses [53, 54]. This fact suggested a role of p53 in the manifestation of oncogenic potential of these viruses. It was found that E6 protein of high-risk HPV (but not of low-risk HPV) also could interact with p53 in *in vitro* systems [55]. However, the biological significance of viral genes interaction with p53 seems to be different in these two systems, since, in the first two cases, the complexes stimulate the stabilization of p53 protein, while in the system with the E6 HPV 16 gene the half-life of p53 decreases. This is explained by p53 protein degradation by the ubiquitin system [56]; this is the major factor in degradation for cytosolic and nuclear proteins in eukaryotic cells [57]. The association of E6 in a complex with one of the components of this system, ubiquitin–protein ligase with molecular mass of 105 kD, referred to as E6-AP, is necessary for p53 degradation by E6 [58].

In addition to the ability to stimulate p53 degradation, E6 protein inhibits several functions of wild-type p53, including transcriptional activation and repression, i.e., it competes for the functions playing the major role in the suppression of malignant growth by p53. It was also shown that E6 increased the level of mutagenesis and genomic instability [59]. Since in the majority of cervical carcinomas (in contrast to many other human tumors) mutations of the p53 gene were not detected [60], presumably the expression of high-risk HPV E6 protein has the same effect on the p53 as the somatic mutations, i.e., the loss of p53-regulated transcription and inhibition of a normal cellular response to DNA damage.

The p53 protein has several other effects that can participate in the process of transformation and are independent of p53. These are activation of heterologous promoters [61], decrease of apoptosis [62], telomerase activation (an enzymatic protein complex maintaining the length of chromosomal telomeres) [63], interaction with a Ca^{2+}-binding protein E6BP identical to ERC 55 protein [64], association with a poorly studied protein-kinase and its substrate [65], and transactivation of α-prothymosin and c-myc [66]. The functional significance of these interactions remains unknown.

E7 gene. The product of the E7 gene is a relatively small phosphoprotein consisting of 98 amino acids. The N-terminal domain of this protein (amino acids 1-38) mainly consists of hydrophilic amino acids, while the C-terminal domain (amino acids 39-98) is more hydrophobic. Although this protein has a calculated molecular mass of 11 kD, HPV 16 E7 protein migrates in polyacrylamide gel electrophoresis as a molecule with molecular mass of 18-20 kD [67]. E7 is structurally and functionally related to the proteins of other DNA-containing viruses, such as the large T antigen of SV40 and E1A early protein of adenovirus. E7 protein can be divided into three main domains—a conservative region 1 (CR1), a conservative region 2 (CR2), and a conservative region 3 (CR3). All three domains are essential for the manifestation of the biological activity of E7 [68].

The CR1 region consists of a short highly conservative amino acid sequence (6-15) bearing homology with the same motif in the CR1 of adenoviral E1A. However, the CR1 regions of these viruses are functionally different: CR1 of E1A can interact with "pocket" proteins, such as p300, and with pRb with less efficiency, while CR1 of E7 does not interact with p300 [69]. Deletions and point mutations in CR1 of HPV 16 E7 lead to a substantial decrease of transforming potential of this gene [70]. The CR2 region of HPV 16 E7 contains a LXCXE domain (amino acids 22-26) responsible for binding of "pocket" proteins (p105 protein of the pRb suppressor gene and related proteins p107 and p130). Any mutations in this region abolish the interaction with pRb and repress the transforming activity of E7 [71]. Low-risk HPV have one common feature: in the CR2-domain the aspartic acid residue in position 21 of HPV 16 E7 is replaced by glycine in HPV 6 and HPV 11 E7. This substitution seems to provide a higher affinity to pRb for E7 of high-risk HPV, as well as the ability to cooperate with activated *ras* oncogene during the transformation of primary rodent cells [72]. Besides the main binding site for pRb in CR2, CR3 probably has another binding site participating in the association with pRb [73].

The C-terminal region of the CR2 domain contains a site for casein-kinase II (CK II) (serine residues in positions 31 and 32). These two serine residues are phosphorylated *in vitro* by CK II. The biological significance of this phosphorylation remains poorly understood, but perhaps this site can play a role in transformation. The substitution of these serines by neutral alanine residues decreases the ability of E7 to cotransform primary cells

together with ras, while the substitution by a negatively charged residue of aspartic acid induces the wild-type phenotype [74]. In addition, phosphorylation of these two serine residues sharply increases the association of HPV 16 E7 protein with the TATA-box-binding protein TBP [75].

The CR3 domain of HPV 16 E7 has a very poor homology with the CR3 domain of adenoviral E1A protein; however, both domains contain CXXC-sequences participating in Zn^{2+} binding [76]. E7 CR3 sequences differ greatly from that of proteins containing zinc fingers. E7 binding to Zn leads to dimerization of E7. This dimerization is probably important for the transforming activity of E7 [77].

The early data concerning E7 localization were contradictory. This could be explained by the fact that this protein is masked in cells and not all its epitopes are accessible for antibodies. All data point to E7 localization in several cellular fractions, mainly in the nucleus and nucleoli [78, 79].

The ability of high-risk HPV E7 for immortalization and transformation depends on the used cell type. The E7 of HPV 16 and HPV 18 can induce the focuses of transformation and growth in semi-solid media in various stable cell lines of rodent fibroblasts, for example in NIH3T3 and Y31 [80, 81]. A lower efficiency of transformation was observed for low-risk HPV E7: the cells did not grow in soft agar, but were found to be tumorigenic for hairless mice [82].

The E7 proteins of HPV 16 and HPV 18 alone are able to induce immortalization of primary rodent cells [83], and its cotransfection with activated ras can induce transformation of these cells [84]. The E7 gene alone induces immortalization in the human keratinocyte system. Its efficiency significantly increases by cotransfection with the high-risk HPV E6 gene. In certain cases this cotransfection may lead to transformation [85]. In addition, the E7 gene can induce immortalization of several other cell types, including breast and ovarian epithelial cells [86, 87]. The E6 and E7 genes of low-risk HPV were not able to immortalize primary keratinocytes [88].

The major function of the high-risk HPV E7 gene is to deregulate the cell cycle machinery mainly by induction of a G_0–S phase transition. This is accomplished by activation of several cellular genes by E7 and by direct E7 interaction with proteins regulating the cell cycle.

Two major steps can be pointed out in the cell cycle: the genome replication and the following cell doubling. These two events are separated by two phases: G_1 before DNA replication and G_2 before mitosis. The appropriate transition of the cycle is controlled by cyclin-dependent protein kinases (CDK). In normal cells the transition from G_0 phase is initiated by growth factors and their receptors specific for a given cell type. At this stage the growth factors activate the expression of D-type cyclins and associated kinases (CDK 4 and CDK 6) inducing the transition of the cells to the G_1 phase. These events are accompanied by the activation of E and A cyclins and of the E2F-1 transcription factor. The proteins of retinoblastoma gene family ("pocket" proteins pRb, p107, p130) block the cell cycle progression by interaction with cellular transcription factors of the E2F and DP families. "Pocket" proteins are inactivated by phosphorylation in many sites by CDK, leading to the formation of transcriptionally active heterodimers E2F/DP. Certain CDK inhibitors, i.e., $p15^{INK\ 4B}$, $p16^{INK\ Y}$, $p21^{WAF\ 1}$, and $p27^{KIP\ 1}$, delay cells in G_1 phase by restriction of pRb and related protein phosphorylation [89].

The E7 gene of HPV 16 ensures the cell transition from G_1 to S phase of the cell cycle and increases the proliferative activity of primary keratinocytes and human breast epithelial cells. The E7 gene can overcome the control of the cell cycle progression at the G_1/S stage, suggesting that E7 is able to neutralize or to overcome the blocking effect of physiological CDK inhibitors such as $p21^{WAF}$ and $p27^{KIP\ 1}$ that are induced by p53 and serum elimination [90].

The HPV 16 E7 gene can interact with a protein of the pRb105 suppressor gene leading to an increase in pRb inactivation [91]. It is accompanied by the induction of activity of cellular transcription factor family consisting of multiple E2F/DP-heterodimers. E7 and E2F-1 bind different sites of pRb [92]. p107, a protein related to pRb, also binds CR2 of E7 [93]. Two different E2F–p107 complexes exist at different stages of the cell cycle, but E7 specifically inactivates the complex specific for the G_1 phase [94].

Among the genes activated by the liberation of E2F, an E2 gene of type 5 adenovirus and the genes coding for b-myb, cyclins A and E were identified [95-97].

In addition to pRb and related proteins associated with cell cycle regulation, some other proteins (regulators of transcription) were found to be E7 targets. An AP-1 family of transcription factors is among these proteins. The transactivation of AP-1-directed genes takes place as a result of the interaction of AP-1 with E7 [98]. Also, E7 of HPV 16 can interact with the TATA-box-binding protein and with a TBP-associated factor, TAF110 [99].

The possibility of physical interaction of HPV 16 E7 with cyclin A and CDK2 through the CR2 domain [100] as well as with cyclin E in complex with CDK2 and p107 was confirmed [101]. Perhaps the interaction of E7 with cyclins is mediated by p107 that, as cited above, can be associated with cyclins A and E [102]. A CDK inhibitor, $p27^{KIP\ 1}$, binds E7 through the C-terminal domain. This leads to the functional inactivation of $p27^{KIP\ 1}$ [103].

One of the specific features of the HPV 16 E7 gene is its ability for proliferation as well as for apoptosis. The latter phenomenon is clearly expressed in normal

human fibroblasts in the absence of E6 gene activity [104]. The pRb-binding domain of E7 is responsible for this activity. Typically the apoptosis induced by E7 develops in mouse cells with deleted p53 gene. Thus, the apoptosis induced by E7 may pass through p53-dependent and p53-independent pathways [53].

Functional cooperation between E6 and E7 oncoproteins. The data presented above point to the oncogenic potential of E6 and E7 *per se*, but this capability significantly increases by their joint expression [41, 85]. This data provides evidence that the viral genes can functionally cooperate in the process of cell transformation. The ability of E6 to disturb the control of reproduction regulation by inhibition of E7-directed apoptosis seems to be the most interesting aspect of this problem. This suggestion was experimentally confirmed on the transgenic mouse model [105], and on uroepithelial human cells [106]. Thus, the functional cooperation between E6 and E7 of high-risk HPV in the transformation process may be partially associated with E6-induced apoptosis inhibition, accompanied by elimination of cells with disrupted system of growth signals including E7-directed ones.

The functions of E6 and E7 are also tightly connected on the level of cell cycle deregulation and cell division. According to this scheme, pRb is a common target for both viral proteins as a result of physical interaction with E7 and the alteration of its phosphorylation level by E6. According to this scenario E6 inhibits $p21^{WAF}$ activation (an inhibitor of cyclin-dependent kinases), mediated by p53 [48]. $p21^{WAF}$ induction by p53 controls non-phosphorylated pRb status essential for pRb function as a controlling factor in G_1 phase of the cell cycle [107]. Inhibition of $p21^{WAF}$ induction (mediated by p53) by p53 may lead to the disturbance of pRb controlling functions and thus cooperate in pRb inactivation after binding to E7.

Thus, the mechanism of the manifestation of papilloma viruses transforming potential seems to be more or less understandable. The virus has two transforming genes. Their expression in the infected cells is controlled by cellular factors. The products of these genes disturb in various ways the functions of genes playing the key roles in the cell cycle and proliferation.

This mechanism explains the processes taking place in the cells *in vitro*, but many open questions still exist in the case of tumor progression in an organism. Why is a long latent period is required in this case? Are there any specific peculiarities in DNA isolated from tumors in comparison to DNA from cells transformed *in vitro*? We have no clear answers to these questions. However, some recent data indicate that the accumulation of additional mutation (secondary genetic changes) takes place in the cell in addition to primary genetic changes induced by the presence of the viral genome. The sum of these changes may lead to the selection of a cell clone and to the appearance of a monoclonal cell population, the division of which cannot be restricted by any of the negative factors. Analysis of microsatellite sequences on the separate chromosomes (the loss of heterozygosity—LOH and the instability of these repeating sequences) provides the additional information about changes in the chromosomes in cervical tumors containing the papilloma virus genome.

SECONDARY GENETIC CHANGES IN CERVICAL TUMORS

The analysis of DNA from cervical tumors for the presence of LOH by microsatellite sequence amplification provided evidence of genomic instability in malignant cells. We should emphasize that the detection of LOH probably indicates the presence of a putative suppressor gene in this region.

LOH was detected in more then 20% of tumors on chromosomes 1, 3, 4, 5, 6, 11, 18, and 20 providing evidence that secondary genetic changes may take place on many chromosomes [108]. The maximal LOH frequency was observed on chromosomes 6p, 3p, and 18 (43, 39, and 35%, respectively) [109]. Subsequent studies revealed the LOH also on chromosomes 4p, 6q, 15q, 18q, and Xq [110]. Four independent regions were found on chromosome 3, three of them mapped on its short arm, where one of the possible suppressor genes, the FHIT gene, is also mapped [111]. Allelic losses were also found on the long arm of this chromosome, suggesting the existence of other suppressor genes [112]. In cervical cancer LOH on chromosome 3 was detected in a region where the β-catenin gene is mapped [113]. β-Catenin is the key component of cellular contacts. Perhaps the high frequency of LOH on chromosome 3 correlates with disease progression [114].

One independent region of possible suppressor genes localization was revealed on each arm of chromosome 4 [115]. One such locus was detected on the pretelomeric region of the short arm of chromosome 5 [116].

A significant genetic instability was found on chromosome 6 [108, 109, 117-119]. The maximal LOH frequency was observed within a region of the short arm, where the gene of the main complex of histocompatibility (HLA) was mapped. The presence of LOH on the long arm suggests the presence of supposing suppressor genes also on this chromosome.

According to different data, the LOH frequency varies between 16 and 88% for the short arm and between 22 and 92% for the long arm of chromosome 11 [114, 120-122], i.e., putative suppressor genes can be localized on this chromosome too.

However, we should emphasize that the LOH frequency on chromosome 13, where one of the main sup-

pressor genes, pRb, is mapped and on chromosome 17, where p53, another suppressor gene is mapped is relatively low and does not exceed 25% [108, 109].

The detected abnormalities in the chromosome structure are specific for cervical tumors and only few of them may be sometimes present in the tumors with other localizations. However these abnormalities are not present in all cervical tumors, suggesting that in every tumor the secondary genetic changes can take place in different regions of the cell genome. Also, we cannot be absolutely sure that the LOH is strictly related to the presence of a hypothetical suppressor gene within this locus. In reality the detected abnormalities concern relatively short regions of the cell genome in the majority of cases. The complete characterization of the regions with deleted sequences seemingly will be hard to realized in the near future.

All of the data presented above could only point to the instability of the tumor cell genome. A significant number of mutations may take place on different chromosomes and only the accumulation of some of them leads to the formation of a cell clone that is completely independent of cell proliferation control.

VACCINATION AGAINST PAPILLOMA VIRUSES AS AN EFFECTIVE TOOL IN IMMUNOPREVENTION OF CERVICAL CANCER

In addition to a significant contribution to the understanding of the molecular mechanisms of transformation from normal cells to a malignant one, cervical cancer has been found to be a neoplastic disease that probably could be prevented by immunoprophylactic means in the near future. This progress is related to the fact that the papilloma viruses are the initiative factors for cervical tumors.

Significant progress in this direction was observed during several recent years. This has led to the creation of vaccines that are on clinical trials today. The indicated data are summarized in the corresponding information from WHO [123].

An actual approach is based on the utilization of so-called "virus-like particles" (VLP) as vaccines. These particles are formed in pro- and eukaryotic cell-free systems expressing a gene encoding the major protein of a viral particle, L1. Self-assembling of this protein followed by the formation of a VLP takes place in these systems. The morphology of the VLP resembles that of an intact virion. Immunization with these preparations induce the production of a high titer of types-specific neutralizing antibodies. It was found that these vaccines prepared for cattle and rabbits effectively prevented corresponding disease after experimental viral infection. These vaccines were inactive in the cases of disseminated infection, and consequently they had no therapeutic effect. Actually, corresponding VLP-vaccines have also been created against human papilloma viruses and WHO has started a vast program of clinical trials and use of these preparations in practice.

Evidently the efficiency of these vaccines could be correctly assessed after 10-20 years, but according to some indirect data we will be able to judge about the benefit of vaccination against HPV already after 5-8 years.

Thus, the data presented above allow several conclusions having general biological significance to be drawn.

1. About 15% of human tumors are caused by viruses.
2. Papilloma viruses are considered as causal agents for cervical cancer.
3. These viruses have specific genes playing a key role in the transformation of normal into malignant cells.
4. The activity of these genes is controlled by viral and cellular factors.
5. Viral transforming genes inactivate the functions of genes playing the key role in cell cycle control and proliferation.
6. Additional mutations are necessary for the formation of a monoclonal cell population and the selection of a clone with uncontrolled proliferation.
7. Vaccines against HPV could be effective in the prevention of cervical tumors.

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