Ph. D.THESIS

Functional analysis of human papillomavirus genomic regions

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2005

INTRODUCTION

Cervix carcinoma is the third most frequent malignant disease in the world; this is one of the leader causes of death in Hungarian women too. Human papillomaviruses (HPVs) have an etiologic role in the development of this cancer: 90 percent of the cervical alterations contain HPV sequences; in a half of these cases HPV 16 is present.

HPV 16 is small, nonenveloped, icosahedral DNA virus belonging to the Papillomaviridae family. The papillomavirus genome contains coding sequences for early (E) and late (L) genes, and a non-coding region (LCR, long control region) with regulating function.

The replicative life cycle of HPV 16 is tightly coupled to the differentiation state of the host keratinocytes. The initial infection with HPV involves undifferentiated basal cells, and the expression of early genes also occurs. Viral genome replication takes place specifically in the differentiated upper epithelial cells, and after the expression of late viral proteins the new virions are established. Since in the differentiated keratinocytes do not progress cellular DNA synthesis, E6 and E7 - the two most important transforming HPV proteins - establish the conditions required to the replication of viral genome, i. e. they retain the host cell DNA in replicative state. Expression of both E6 and E7 is necessary and sufficient for effective immortalisation of primer keratinocytes, therefore resulting outgrowth of immortalized clones that are resistant to terminal differentiation. E6 protein binds and induces ubiquitin-dependent proteolysis of p53 through a mediator protein (E6-associated protein, E6AP), displaying its transforming activity in this way. E7 has been shown to bind to the hypophosphorylated form of retinoblastoma protein (Rb), and prevent it from binding to the E2F transcription factor. As a result of free E2F cells can enter the S phase, consequently they can be immortalised. The expression of these two proteins is under control of P_{97} main early promoter localized in the LCR region of HPV 16 genome. In this regulating region the viral replication origin, binding sites of viral E1 and E2 proteins and sequences specific for different cellular transcription factors can be found.

The mucosal HPVs are subdivided into “low-risk” and “high-risk” types according to their oncogenic potential. HPV6 and HPV11 belong to the former category, while HPV 16, HPV18, HPV31 and HPV45 are classed in the high-risk group. HPV 16 can be divided into six geographically clustered groups, the European group (E), two African groups (Af1 and Af2), and the Asian (A), Asian American (AA) and North American (NA1) variants. These intratype variants have different oncogenic potential: the AA and NA1 associate more

frequently to higher risk neoplastic alterations than other variants. The DNA of HPV persists mostly extrachromosomally in precancerous lesion, while in case of malignant alterations it frequently integrates into the host cell genome. The integration is accompanied at most times by disruption of circular viral genome in E2 gene. Thus, elimination of the E2-mediated negative control of the P_{97} promoter potentially leads to increased expression of the E6 and E7 oncoproteins including enhanced immortalizing capacity. Certain HPV 16 variants from cervical cancers were found to have deletions of variable length in the long control region with consequent elevated transcriptional activity. Detailed examination of different intratype, or natural deletion variant can explain this enhanced transcriptional activity, and may have prognostic value to the outcome of HPV-associated cervical alterations.

In addition to HPV infection, cellular factors (cytokines, growth factors, hormones, immunological and genetic alterations) also contribute to development of cervical cancer. Transforming growth factor-β2 (TGF-β2) is a member of TGF-β superfamily. This cytokine family include more than 40 proteins that have a multiple role in embryonic morphogenesis and differentiation, wound healing, apoptosis, angiogenesis, cellular adhesion and migration. Deregulation of TGF-β signalling is implicated in the pathogenesis of many disease including arthritis, atheroscerosis, glomerulonephritis, human hereditary telangiectasia, and carcinogenesis. Each isoform of mammalian TGF-β is expressed in a tissue-specific fashion, and their effects on cell proliferation can be positive or negative depending on the cell type and cell cycle phases. TGF-β1 is expressed in endothelial, haematopoietic, and connective tissue cells; TGF-β2 in epithelial and neuronal cells; and TGF-β3 primarily in mesenchymal cells. TGF-β plays a dual role in carcinogenesis. In normal cells, TGF-β acts as a tumour suppressor by inhibiting cell growth or by promoting cellular differentiation or apoptosis through inducing cell cycle inhibitor proteins ($p15^{INK4B}$, $p21^{CIP1/WAF1}$). In response to elevated TGF-β levels, the tumour cells become more migratory and invasive, and tumour angiogenesis is also stimulated. During the early stages of carcinogenesis, epithelial cells maintain their sensitivity to the growth inhibitory control of TGF-β, whereas in the late stages this sensitivity is irreversible lost. TGF-β2 is induced during keratinocyte differentiation, and inhibits the proliferation of these cells. Since human papillomaviruses require host cell proliferation to viral life cycle, the antagonistic effects of TGF-β decrease the probability of HPV infection.

It has been recently shown that HPV 16 E6 and E7 proteins reduce the expression of TGF-β2 in cervical keratinocytes. However, the exact molecular mechanism of this inhibitory effect is not known.

AIMS

In the first study we focused on HPV 16 natural deletion variants isolated from cervix carcinoma samples and addressed the following issues:

Whether have these mutations any effect on the transcriptional activity of deletion variants?

Is there any difference among the transcriptional activity of intratype variants?

In the second study we analysed the association between HPV 16 and human transforming growth factor β2 (TGF-β2), which plays an important regulatory role in the differentiation of epithel cells, thus the following issues were addressed:

Have the HPV 16 E6 and E7 genes direct effect on the activity of the TGF-β2 promoter?

Whether is this effect associated to a distinct part of TGF-β2 promoter?

Is there any relation between the effect of HPV 16 oncoproteins and the presence of p53 or retinoblastoma tumour suppressor protein?

Which cellular proteins mediate the observed effect?

MATERIALS AND METHODS

Polimerase chain reaction (PCR), plasmids, cloning

The long control region (LCR) of full-length variants and its deletion counterparts were amplified from HPV 16 positive cervical cancer sample number 33 and number 936. We use Pwo polymerase to all PCR reactions, because this enzyme possess 3'-5' exonuclease (proofreading) activity in addition to its 5'-3' DNA-polymerase activity, resulting increased fidelity of DNA synthesis. The amplification products were purified with the Qiaquick PCR purification kit (Qiagen, USA), digested by restriction enzymes, purified again and cloned into pUC19 vector digested by the same restriction enzymes. The reporter vector pALuc-pt16LCR was constructed using a similar strategy but here the reference full-length HPV 16 clone was used as template in the PCR. The resulting clones were sequenced, and were subcloned into the promoterless luciferase reporter vector pALuc after purifying and restriction cleavage. In order to construct the reporter vector pALuc-16P, the P_{97} early promoter region (nt 14-104) was amplified. After amplification of tissue-specific enhancer region (nt 7527-7854) it was cloned into pALuc-16P in front of the promoter region resulted construct pALuc-16PEN. The reporter construct pC18Sp1-Luc contains 4 E2 binding sites, 2 Sp1 binding sites, and the firefly luciferase gene driven by the adenovirus major late promoter, and it can be efficiently transactivated by papillomavirus E2 proteins. The pCMV1-pt16E2 expression vector contains the full length HPV 16 E2 gene cloned into the pCMV1 eukaryota expression vector.

We obtained the reporter vectors pβ2-528, and pβ2-77 containing various fragments of the human TGF-β2 promoter cloned into pGEM4-SV0CAT. After restriction cleavage we subcloned the promoter fragments into the multiple cloning site of the pALuc luciferase reporter vector. The other fragments of the TGF-β2 promoter were created by PCR using pALuc-B2-528 as template and cloned into pALuc resulting in the reporter constructs pALuc-B2-445, pALuc-B2-352, pALuc-B2-251, and pALuc-B2-183. The pGL2-Prom-B2-161 reporter construct was created by amplifying an upstream TGF-β2 promoter region (from -536 to -376 relative to the transcriptional start site) and cloned into the pGL2-Promoter luciferase reporter vector in front of the SV40 promoter. HPV 16 E6 or E7 coding sequences were cloned into the eukaryotic expression vector pcDNA3.1+ after amplification, restriction cleavage and purifying.

We used pAdE2luc plasmid to examine the expression of HPV 16 E6 and E7 proteins, pcDNA-E2F1 construct expressing E2F-1 transcription factor, and plasmids expressing wild-type or mutant E7 proteins (p16E7Mo, p24Gly, p26Gly, p31Arg, p58Gly, and p91Gly) in the transient transfection experiments.

Sequencing

The LCR variants were sequenced by T7 Sequencing kit. After denaturation with NaOH, precipitation with sodium-acetate and ethanol, primers specific for the pUC19 were hybridizated to the template DNA. The labelling reaction was executed with T7 DNA polymerase and α^{32}S dATP. After that the samples were loaded on 6% acrylamide/7M urea/1xTBE polyacrylamide gel. Following gel dehydration and 16-24 hour exposition the image was developed and fixed.

Verification of the sequences of TGF-β2 promoter constructs, and the pcDNA-16E6 and pcDNA-16E7 was carried out by Abi PRISM BigDye Cycle Sequencing kit. For the sequencig PCR we used 0,4 μg template DNA, 0,02 μg primer and 8 μl TRR mix. After purification of the PCR product by Dye-Ex Spin kit and lyophilization we denaturated the dissolved DNA and sequenced with ABI PRISM 3100 Genetic Analyzer.

Cell culture, transient transfection and luciferase test

HeLa (ATCC CCL-2) cervical adenocarcinoma cells, NIH/3T3 (ATCC CRL-1658) mouse embryo fibroblast cells, and C-33A (ATCC HTB-31) cervical carcinoma cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum, 2 mM L-glutamine and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). At approximately 70% confluence, cells were transfected with a total of 3 or 4 μg of DNA, which included 2 μg of the reporter vectors and 1 or 2 μg of different expression vectors using LipofectAmine Plus (GIBCO BRL) (for HeLa and NIH/3T3 cells) or the calcium-phosphate method (for C-33A cells). The cells were harvested 48 h after transfection by the addition of Reporter Lysis Buffer and one freeze-thaw cycle. The Luciferase Assay System of Promega was used to measure the luciferase activity of cell extracts. In the presence of ATP or coenzyme A luciferase enzyme of the firefly induces photon emission with its substrates in oxidation reaction. Our reporter constructs contain different sequences in

front of the luciferase gene, which influence its transcription. Alterations in the activity of luciferase gene can inform us about the effect of these sequences on the transcription. In the first article we used β-galactosidase test to standardise the luciferase activity. In the second article the β-galactosidase tests were unsuitable to standardise the luciferase results, because we found that the β-galactosidase reporter constructs containing viral promoters (RSV-βGal and SV40-βGal) were trans-activated by HPV 16 E6. Instead, the Bradford protein assay was performed to standardize for protein concentration of the cell extracts. Each transfection experiment was performed independently at least 3 times.

RESULTS AND CONCLUSIONS

Transcriptional activity of human papillomavirus type 16 variants having deletions in the long control region

During a previous study, we isolated HPV 16 LCR variants from cervical cancer samples. After amplification of the LCR region and electrophoresis, in addition to bands corresponding to the full-length LCR, some shorter bands were also found in a few cases. In this study, two samples (no. 33 and 936) with episomal HPV 16 genomes were chosen for detailed analysis of these putative deletion variants. On the basis of sequence analysis, the HPV 16 genome in the sample no. 33 is classed among the Asian-American variant group, while in the sample no. 936 the HPV 16 belongs to the European prototype. One variant (936LCRΔ1) contained an insertion of ´TA´ just after nt 7521 and a 211 bp deletion (nt 7522 – nt 7732) in the tissue-specific enhancer region. The other variant (936LCRΔ2) had a 378 bp deletion (nt 7564 – nt 35) encompassing a great part of the enhancer and both 3´ silencer regions (the YY1 dependent and the CDP/Cut dependent), and the Sp1 binding site in the promoter region. From the other case (33), again a full-length clone (33LCR) and two deletion variants were isolated. A large part of the enhancer region and the total YY1-dependent silencer region were deleted (292 bps, nt 7566 – nt 7857) in isolate 33LCRd1. The other deletion variant (33LCRΔ2) contained a 563 bp deletion (nt 7363 – nt 19) encompassing the whole enhancer and both 3´ silencer regions (Fig. 1).

In addition to these deletions, we found point mutations (compared with the reference HPV 16 sequence) in the binding sites of GRE, TEF-1 and NF1 transcription regulating factors, but these alterations are also present the variants with full length LCR. Among single nucleotide alterations in the 33LCR the 7729 A/C has significant importance: this polymorphism is often found in the higher oncogenic risk Asian-American variants, and it is thought to be responsible for enhanced transcriptional activity of the LCR region of AA variants.

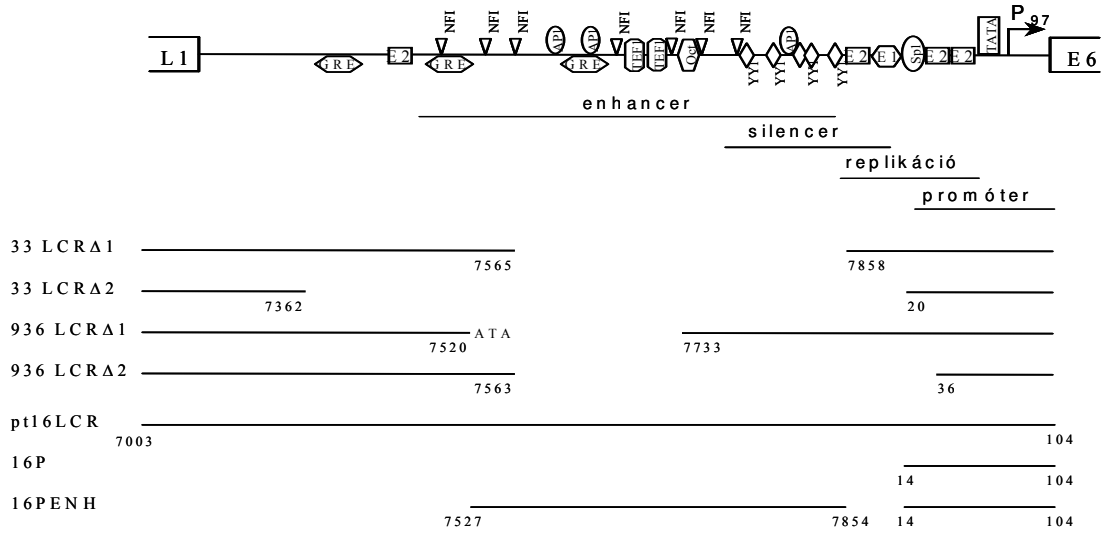

Figure 1.
Location of deletions in LCR,
and schematic representation of the reporter vectors applied in first article

In accordance with previous results, the full-length Asian-American LCR variant 33LCR had higher transcriptional activity than the European variants pt16LCR or 936LCR. The variants 33LCRΔ1 and 33LCRΔ2, which deleted a large part of the enhancer region and one or both silencer regions had still significantly higher activity than the construct containing the P_{97} promoter only (pALuc-16P). This was very impressive in the case of 33LCRΔ2, which had the largest deletion but carried out the highest transcriptional activity of all deletion variants tested. In addition to the P_{97} promoter region (nt 20 – 104), 33LCRΔ2 contains only the 3´ part of the L1 gene and a 210 bp upstream LCR element (nt 7003 – 7362). We hypothesise that this 5' LCR segment of the HPV 16 genome contains a transcriptional enhancer region similar to those described for HPV 11 and 18. This region can provide transcription of the E6/E7 oncogenes in the absence of the central epithelial-specific enhancer region. This 5'-LCR segment of HPV 16 was shown to represent a nuclear matrix attachment region (MAR) and to function as either a repressor or activator of the P_{97} promoter depending on the physical state of the viral DNA. This 5'-LCR MAR was shown to function as a silencer when the reporter vector was present in an episomal form, while it functioned as an enhancer when the vector had been integrated in the host genome. In our study, the reporter vectors were tested in transient transfections (when they are present episomally), and still the 5'-LCR seemed to function as a weak enhancer. It should be considered that the function of this LCR segment was studied in the presence of the central enhancer region, while in the variants tested here, this enhancer region was deleted partly or completely. This may explain the partly

different results found in the two studies. One possible transcription factor responsible for it may be AP1, which have potential binding sites in this 5' LCR region (at nucleotides 7305, 7332 and 7361). However, there are alternative explanations for the relatively high transcriptional activity of the deletion variant 33LCRΔ2. One such scenario may be that the large deletion in this variant altered the nucleosomal organisation of the LCR and relieved transcriptional repression by nucleosomes, CDP, and the histone deacetylase enzyme HDAC1. It was an interesting finding of this study that parts of the replication origin of the episomal HPV 16 genome (which overlap the CDP-dependent silencer region) were found to be deleted in two variants isolated from specimens with episomal HPV genomes. It is theoretically possible that these deletion variants, which were present in small amounts in addition to the full-length variants, were integrated into the host chromosome and replicated with in the cancer cells where they were isolated from. Alternatively, other sequences (perhaps, outside the LCR) may also function as origins of replications of the episomal viral genome.

In variant 33LCRΔ2, 2 of the 4 binding sites for the viral replication/transcription factor E2 were deleted. We wanted to see if expression of HPV 16 E2 had any specific effect on this variant different from that on the full-length clone. The low level of E2 protein increases the amount of E6/E7 mRNA through the P_{97} promoter, while at high level it inhibits the activity of these genes. The repressive effect of E2 protein on the P_{97} promoter realizes through two proximal E2 binding sites by removing the positive-acting Sp1 transcriptional factors. The role of E2 also depends on the physical state of HPV genome, because the circular viral DNA is often disrupted in E2 gene during integration. Consequently the P_{97} promoter is released from the negative effect of E2, and the transcription of E6 and E7 oncoproteins increase. Since the sample no. 33 and no. 936 contain the HPV 16 episomally, the E2 promoter could have remained active in the viral genome. In cotransfection experiments overexpression of E2 had little effect on the transcriptional activity of the control reporter vector pt16LCR in HeLa cells. In contrast, E2 inhibited transcription modestly from the variants 33LCR and 33LCRΔ1 and strongly from 33LCRΔ2. It should be emphasised that all 4 E2 binding sites are conserved in 33LCR and 33LCRΔ1, while 2 promoter-distal E2 binding sites are deleted in 33LCRΔ2. On the other hand, the two promoter-distal E2 binding sites are thought to have positive effect on the P_{97} promoter, while the two binding sites within the promoter region (which are retained in 33LCRΔ2) were shown to inhibit the

activity of the promoter. Thus, it is not surprising that deletion of the two promoter-distal E2 binding sites led to an increased inhibition of the P_{97} promoter by E2.

Effect of human papillomavirus type 16 E6 and E7 oncogenes on the activity of the transforming growth factor-β2 (TGF-β2) promoter

Recently, it was shown *in vitro* that HPV 16 E6 and E7 decrease the expression of TGF-β2 mRNA and the secretion of biologically active TGF-β2 protein in primary human keratinocytes. However, it was not investigated whether this is accomplished by a direct effect on the TGF-β2 promoter or by some other mechanisms. Owing to the fact that this decrease of TGF-β2 secretion can be mediated by p53 and retinolastoma protein, our transient transfection experiments were performed in three different cell cultures. C-33A is an HPV-negative cervical carcinoma cell line containing mutated and functionally inactive p53 and pRb (retinoblastoma) proteins. HeLa is an HPV18 positive cervical adenocarcinoma cell line, in which the p53 and pRb proteins are not mutated, but are thought to be functionally inactivated by the HPV oncoproteins E6 and E7, respectively. NIH/3T3 is an immortal mouse fibroblast cell line containing wild-type p53 and pRb proteins. First, we tested the effects of HPV 16 E6 and E7 on the transcriptional activity of the TGF-β2 promoter constructs pB2-77 and pB2-528 in the different cell lines. In C-33A cells, HPV 16 E6 and E7 had weak trans-activating effects on the transcriptional activity of the pB2-77 or the pB2-528 construct. In HeLa cells, over-expression of E6 strongly trans-activated both the pB2-77 and the pB2-528 reporter constructs. Over-expression of E7 had no effect on pB2-77, while it resulted in a moderate trans-activation of pB2-528. Expression of the E6 protein resulted in weak trans-activation of pB2-77 and pB2-528 in NIH/3T3 cells. Expression of HPV 16 E7 slightly trans-activated the minimal promoter construct pB2-77, while it caused a significant (3-4-fold) down-regulation of pB2-528, which contains more upstream transcriptional regulatory elements than pB2-77. We found that even a small dose of HPV 16 E7 strongly repressed the activity of the pB2-528 reporter construct. Here, expression of E7 had a positive effect on pB2-77 only at the highest concentration used, which may indicate that the positive effect of E7 on the minimal TGF-β2 promoter may be not biologically significant. Although a recent work also found HPV 16 E6 to down-regulate the expression of TGF-β2 mRNA, E6 failed to repress TGF-β2 promoter activity in our study. This suggests that the down-regulation of

TGF-β2 mRNA level by HPV 16 E6 may not be explained by the repression of promoter activity, but rather by some other mechanism.

Next, we tried to determine which part of the TGF-β2 promoter is responsive to the inhibitory effect of HPV 16 E7. To this end, a deletion series of promoter constructs were created by PCR containing TGF-β2 promoter fragments of variable length all extending to nt +57 relative to the transcriptional start site. The shorter promoter constructs pB2-77, pB2-183 and pB2-251 were more or less activated by E7 in NIH/3T3 cells. Transcription from pB2-352 was down regulated by E7, although the activity of this construct was very low already in the absence of E7, perhaps due to the presence of a transcriptional silencer. HPV 16 E7 had no significant effect on the transcriptional activity of pB2-445, while it significantly repressed the activity of pB2-528, in accordance with the results described above. It can be concluded from these data that the repressive effect of HPV 16 E7 on the transcriptional activity of the TGF-β2 promoter can be localized to the promoter region - 528 to - 251 relative to the transcriptional start site. We also showed that this transcriptional regulatory region could confer sensitivity to E7-mediated inhibition after placing it directly in front of a heterologous promoter such as SV40. This indicates that the upstream E7 responsive negative regulatory element described here functions not only in the context of the TGF-β2 core promoter, and its function is not dependent on a specific distance between the E7 responsive element and the core promoter or on intervening transcriptional regulatory elements.

In order to verify the specific effect of HPV 16 E7 on the TGF-β2 promoter, and to see whether this effect depends on the ability of E7 to bind pRb or related 'pocket' proteins, we made use of HPV 16 E7 mutant constructs. The ability of the HPV 16 E7 mutants to repress the TGF-β2 promoter was tested in transient transfection assays performed in NIH/3T3 cells. We found that an E7 mutant (p24Gly) that is unable to bind to pRb or to the related 'pocket' proteins p107 and p130 lost the ability to repress the TGF-β2 promoter. Another mutant, which is unable to bind to pRb but is able to bind to p107 and p130 (p26Gly) was also unable to repress the TGF-β2 promoter. Together, these results indicate that the binding capacity of E7 to pRb is indispensable in the repression of the TGF-β2 promoter. The mutants encoded by p31Arg, p58Gly and p91Gly retained only weak (2 to 3.5-fold) repression activity on the TGF-β2 promoter compared to the wild-type construct, suggested that the casein kinase II phosphorylation site and the Cys-X-X-Cys motif do not have significant role in the inhibition of TGF-β2 promoter.

One possible mechanism how E7 represses the TGF-β2 promoter may be to release transcriptionally active E2F from pRb/E2F complexes. If this assumption is true, over-expression of E2F must substitute HPV 16 E7 in the repression of the TGF-β2 promoter. In order to test this hypothesis, we transfected NIH/3T3 cells with the reporter vector pB2-528, along with an expression vector containing full length E2F-1 or empty vector as a control. The results of luciferase tests indicated that over-expression of E2F-1 in NIH/3T3 cells resulted in a 2.3-fold reduction of TGF-β2 promoter activity, which is comparable to the effect of E7 expressed from the same expression vector. These results suggest that HPV 16 E7 may repress the transcriptional activity of the TGF-β2 promoter by binding to pRb and releasing E2F from the pRb/E2F complex. However, the upstream TGF-β2 promoter region shown here to be responsible for E7 repression has not been previously characterized, and we found no consensus E2F binding sites there. Therefore, E2F may exert its effect not by direct binding to the TGF-β2 promoter but rather by some indirect mechanism.

According to published data over-expression of E2F1 is able to overwrite the G_1 arrest of cell cycle caused by TGF-β. The activity of cyclin E is increased by E2F1, and as a result of this cdk2 phosphorylate Smad3, which plays a significant role in TGF-β signalling pathway. Owing to the phosphorylation, the transcription activity of Smad3 decreases, and so does the level of p15 cyclin-dependent inhibitor, consequently the cell cycle is shifted to enter S phase. Our data reveal the same mechanism. If the E2F1 released by HPV 16 E7 protein from pRb/E2F complex decreases the level of TGF-β2, the transcription of Smad3-target genes is inhibited, the level of cdk2 and cdk4 increases respectively, and consequently cells can enter the S phase.

SUMMARY

In the first study we examined some HPV16 clones with apparently long deletions in their LCR isolated from patients with cervical cancer. The deletion variants contain deletions in the tissue-specific enhancer region, or YY1-dependent and CDP/Cut-dependent silencer elements. In addition to these deletions, we found point mutations (compared with the reference HPV16 sequence) but these alterations are also present in the variants with full length LCR. In accordance with previous results, the full-length Asian-American LCR variant had higher transcriptional activity than the European variants. In general, the deletion variants have reduced activity compared to full-length variants. In the case of a deletion variant, which had the largest deletion, we measured the highest transcriptional activity of all the deletion variants tested. In addition to the P_{97} promoter region, this variant contains only the 3' part of the L1 gene and a 210 bp upstream LCR element. We hypothesise that this 5' LCR segment of the HPV 16 genome contains a transcriptional enhancer region, which has not been previously characterized.

In our following study the effect of the HPV 16 E6 and E7 oncoproteins was examined on the human transforming growth factor β2 (TGF-β2), which plays an important regulatory role in the differentiation of epithel cells. The HPV 16 E6 had trans-activating effects on the TGF-β2 promoter, which was independent of p53, and carried out through TATA-box present in the TGF-β2 promoter. Expression of HPV 16 E7 caused a significant (3-4-fold) down-regulation of TGF-β2 promoter in the NIH/3T3 cells, which contain biologically active retinoblastoma protein (Rb). The repressive effect of HPV 16 E7 on the TGF-β2 promoter could be localized to the promoter region - 528 to - 251 relative to the transcriptional start site. Our experiments performed with E7 mutants showed that binding of E7 to pRb is absolutely necessary to inhibit the TGF-β2 promoter. Application of pcDNA-E2F1 construct expressing E2F-1 transcription factor yielded a 2.3-fold reduction of TGF-β2 promoter activity, which is comparable to the effect of E7 expressed from the same expression vector. All these results suggest that HPV 16 E7 may repress the transcriptional activity of the TGF-β2 promoter by binding to pRb and releasing E2F from the pRb/E2F complex.

PUBLICATIONS

Articles serve as a basis of this thesis

I. G. Veress, M. Murvai, K. Szarka, A. Juhász, J. Kónya, L. Gergely
Transcriptional activity of human papillomavirus type 16 variants having deletions in the long control region
Eur. J. Cancer., 37: 1946-1952 (2001) IF: 3,694

II. M. Murvai, A. A. Borbély, J. Kónya, L. Gergely, and G. Veress
Effect of human papillomavirus type 16 E6 and E7 oncogenes on the activity of the transforming growth factor-β2 (TGF-β2) promoter
Arch. Virol., 149: 2379-2392 (2004) IF: 1,876

Other articles

G. Szládek, A. Juhász, L. Asztalos, K. Szőke, M. Murvai, K. Szarka, G. Veress, L. Gergely, J. Kónya
Persisting TT virus (TTV) genogroup 1 variants in renal transplant recipients.
Arch. Virol.,. 148: 841-51. (2003) IF: 1,876

Presentations and posters

G. Veress, K. Szarka, M. Murvai, J. Kónya, L. Gergely.: Transcriptional activity of human papillomavirus type 16 variants having deletions in the long control region
First Joint Meeting of the Slovenian Society for Microbiology and the Hungarian Society for Microbiology, August 24-26. 2000, Keszthely

M. Murvai, Á. A. Borbély, G. Veress, L. Gergely.: Effect of human papillomavirus type 16 E6 and E7 oncogenes on the activity of the transforming growth factor-β2 (TGF-β2) promoter
Jubilee Congress of the Hungarian Society for Microbiology, October 10-12, 2001, Balatonfüred

M. Murvai, Á. A. Borbély, K. Szarka, L. Gergely, G. Veress: Effect of human papillomavirus type 16 E6 and E7 oncogenes on the activity of the transforming growth factor-β2 promoter
20th International Papillomavirus Conference, October 4-9. 2002., Paris, France

M. Murvai, Á. A. Borbély, L. Gergely, G. Veress.: Relations between E-cadherin tumour suppressor gene polymorphism, human papillomavirus (HPV) and cervix carcinoma
Congress of the Hungarian Society for Microbiology, October 7-9., 2004., Keszthely