PhD THESIS

# ANALYSIS OF THE RELATIONSHIP BETWEEN HUMAN PAPILLOMAVIRUS AND THE CELLULAR SURVIVIN GENE

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2006

## Introduction

Carcinoma of the uterine cervix is one of the most common malignant diseases among women worldwide. Several oncogenic, or high-risk human papillomaviruses (HPV) – 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70 – are thought to play a critical role in the development of the carcinoma. More than 90 percent of the cervical lesions contain different types of HPV, in half of the cases HPV 16 is detected.

Belonging to the Papillomaviridae family HPV 16 is a small (55 nm), nonenveloped, icosahedral DNA virus. The virion particles consist of a single molecule of a double-stranded, circular DNA approximately 8000 bp in size. The genom of the virus is divided into an early (E) region, which encodes the genes required for viral DNA replication and cellular transformation, a late (L) region that codes for the capsid proteins and a non-coding region regulatory region (LCR, long control region).

The two most important transforming HPV proteins, E6 and E7 oncoproteins are involved in the immortalisation of the infected primery keratinocytes. The E6 protein transcriptionally activates or represses several cellular and viral promoters. Through a mediator protein E6AP (E6-associated protein) E6 is able to bind to and induce ubiquitin-dependent proteolysis of the tumour suppressor protein p53. It also influences the binding of p53 to it's target genes resulting in inhibition of the p53-dependent transcription. p53 mediates cell cycle arrest in the G1-S phase as a response of DNA damage or other kinds of stress. Thus, cells are provided time for DNA repair mechanism, or in case of severe DNA damage p53 promotes apoptosis. Since E6 is able to bind to and mediate the degradation of p53, it may have a role in the inhibition of apoptosis.

E7 has been shown to bind to the hypophosphorylated form of the retinoblastoma protein (Rb). The active hypophosphorylated form of Rb binds to the members of the E2F family of transcription factors and brings about the repression of E2F-dependent genes, thus blocking the the entry into S phase of the cell cycle. When cells start to divide, Rb is hyperphosphorylated, and E2F proteins are released from the binding. As E7 is able to bind to the hypophosphorylated form of Rb, this binding disrupts the complex between Rb and E2F, which allows the transcription of E2F-dependent genes whose products are required for the cell to enter the S phase of the cell cycle. E7 oncoproteins of high-risk HPVs are not only able to bind to the Rb protein but to induce the ubiquitin-dependent degradation of it.

More than 200 human papillomavirus types have been recognized on the basis of DNA sequences, 85 of them are well characterized, and an additional 120 isolates are

potentially new genotypes. Most HPVs can be classified into one of three groups: cutaneous types cause nongenital skin warts in otherwise normal individuals, the EV types causing nongenital skin lesions primarily in individuals with epidermodysplasia verruciformis (EV), and the genital-mucosal types infecting the genital skin, genital mucosa, or nongenital mucosa. The genital-mucosal types are further subdivided into high-risk and low-risk types. Low-risk types, such as HPV 6 and HPV 11 are more frequent in benign lesion – such as condyloma acuminatum – while high-risk types (HPV 16, 18, 31, 33) are mostly izolated from malignant lesions of the lower genital tract, primarily from cervical carcinoma and it's precancerous stages.

Before tumours appear, HPVs often persist without any symptom in the mucosa of the cervix for years or even decades. Invazive cervical carcinoma develope through different precancerous stages. Considering the long period between HPV infection and the development of the disease, other factors may play a role in the development of tumour besides the presence, type and features of the virus. These factors include the genetical and immunological characteristics of the host, cellular processes, different co-factors, such as hormonal effects, smoking, nutrition, sexual habits.

A potential co-factor may be a recently discovered cellular protein, called survivin, a novel member of the inhibitor of apoptosis protein (IAP) family. Survivin is strongly expressed in embryonic and foetal organs, but is undetectable in most terminally differentiated normal adult tissues. The product of the gene is abundantly re-expressed in the majority of human tumours, as in cancer of the uterine cervix. Besides the caspase-dependent inhibition of apoptosis, it has a role in the regulation of the cell cycle and cell division. Survivin is expressed in a cell cycle regulated manner with a peak in the G2/M phase of the cell cycle, – when it is associated with the microtubules of the mitotic spindles – and a rapid down-regulation in G1 phase. This is largely controlled at the transcriptional level and mediated by cell cycle-dependent elements (CDE) and cell cycle homology regions (CHR) located in the proximal region of the survivin promoter. The presence of CDE and CHR are characteristics for G2/M phase genes. Several single nucleotide polymorphisms (SNP) were identified within the promoter region of the survivin gene, one of which is located at the CDE/CHR repressor binding site (–31 to the first nucleotide of the ATG start codon). This polymorphism (G/C) seemed to be associated with over-expression of survivin at both mRNA and protein levels, and changed cell cycle-dependent transcription by the functional disruption of binding at the CDE/CHR repressor motifs in a number of cancer cell lines.

HPV 16, like other high-risk human papillomaviruses, is able to infect the proliferating, basal keratinocytes of the cutan and mucosa but replication of the viral genom is only possible in the differentiated upper epithelial cells where cell cycle is already arrested, and no DNA synthesis takes place. Since the maintenance of the proliferation of the host cell is essential for viral replication, the virus modulates cellular processes, stimulates proliferation and inhibits apoptosis. These events may result in the accumulation of mutations and the development of malignant tumours.

The over-expression of survivin may have a role in HPV induced cervical carcinogenesis. Unlike in normal tissues, survivin is re-expressed in human tumours, such as cancer of the uterine cervix. It inhibits apoptotic processis resulting in continuous proliferation. The role of survivin in tumour development is feasible, but the molecular and cellular mechanisms that leads to the dysregulation of survivin expression, is only partially revealed. The expression of survivin was shown to be negatively regulated by the cellular tumour suppressor protein p53. As the HPV 16 E6 oncoprotein is able to induce the degradation of p53, we assumed that it might have an effect on survivin transcription. Moreover, the expression of survivin was shown to be regulated by other viral and cellular oncogenes. Thus, it is possible that HPV 16 oncogenes have effects on the expression of the human survivin gene.

**Aims**

In our studies we addressed the following questions:

I. Do the HPV oncoproteins 16 E6 and E7 have any effect on the expression of the human survivin gene?

Is there any relation between the effect of HPV 16 oncoproteins and the presence of cellular proteins, or the cell cycle?

Do the oncogenes of HPV 16 have an effect on the endogenous survivin mRNA level?

II. What is the rate of the genetic polymorphism located in the CDE/CHR repressor elements of the human survivin promoter (at the –31 nt relative to the ATG start codon) in cervical cancer patients, P3 (equivocal cytologic atypia) population and in a control group.

Does the mentioned survivin promoter polymorphism have any role in the development of HPV-associated cervix carcinoma?

## Materials and methods

*Plasmid constructs, cell cultures and retroviral transduction*

The luciferase reporter constructs pLuc-230c, pLuc-441c, pLuc-1430c and pLuc-2840c contain different fragments of the human survivin promoter. The HPV 16 oncoproteins were expressed by the pcDNA-16E6 and pcDNA-16E7 expression vectors. The plasmid encoding wild-type HPV-16 E6 (pJ4Ω-16E6) and its deletion mutant derivatives (pJ4Ω-16E6Δ106-110, -16E6Δ111-115 and -16E6Δ128-132) were used as well. The reporter construct p53CON-Luc contains a consensus p53-binding site cloned into the luciferase reporter vector pGUP.PA.8. The expression vector pcDNA3-p53Pro carries the codon 72 proline variant of human p53. For the transient transfections HeLa HPV-18-positive cervical adenocarcinoma cell line, MCF-7 human breast cancer cell line and Saos-2 human osteosarcoma cell line were used. Human embryonic fibroblast (HEF) cells were transduced by recombinant retroviruses produced by PA317-LXSN, -16E6, -16E7 and -16E6E7 cell lines.

Amphotropic retroviruses produced by the packaging cell lines were titrated on HeLa cells using G418 (500 μg/ml) as a selective agent and counting the number of drug-resistant colonies after methylene blue staining. HEF cells were transducted by the different retrovirus vectors and selected in media containing 200 μg/ml G418. The derived cell lines are named as follows: HEF-LXSN, HEF-16E6, HEF-16E7, HEF-16E6E7.

*Transient transfection and luciferase test*

HeLa and MCF-7 cells were transfected with 2 μg of the reporter vectors and 1 μg of different expression vectors by using Lipofectamine 2000. Saos-2 cells were transfected with 5 μg of the reporter vectors together with 2 μg of the expression vectors by electroporation (950 μF, 300 V) using GenePulser II (Bio-Rad). 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. The Bradford protein assay was performed to standardize the protein concentration of the cell extracts.

*Synchronization of the cell cycle*

For G1 arrest, HEF-derived cell lines were treated with aphidicolin (10 μM) for 18 h and then for a further 24 h. For G2/M arrest, cells were treated with aphidicolin (10 μM) for 18 h and then released into nocodazole (0,2 μg/ml) for 24 h.

In the case of HeLa cells, G1, S and G2/M arrest were induced by treating the cells with 10 μM aphidicolin, 2 mM thymidine or 0,2 μg/ml nocodazole for 24 h, respectively.

*Flow cytometric analysis*

For analysis of the cell cycle, cells were harvested by trypsinization, washed in PBS, fixed in 70% ethanol, stained (in PBS with 20 μg/ml propidium iodide, 200 μg/ml RNase A and 0,1% Triton-X 100) for 30 min and analysed for DNA content on a FACScan (Beckton Dickinson) cytometer. In order to assess the distribution of cells within the cell cycle, the ModFit LT software (Beckton Dickinson) was used.

*Semi-quantitative RT-PCR*

Total cellular RNA was prepared from the cell lines by using TRI reagent according to the manufacturer's protocol. The DuraScript RT-PCR kit (Sigma) was used to prepare cDNA. For reverse transcription, the antisense primers (table 1.) used for amplification of survivin, HPV-16 E6, E7 and GAPDH cDNA were used.

| | Sense és antisense primerek szekvenciái |
|---|---|
| survivin | 5'-GGCAGCCCTTTCTCAAGGACCACC-3'<br>5'-GATGGCACGGCGCACTTTCTTCGC-3' |
| HPV 16 E6 | 5'-TGTTTCAGGACCCACAGGAG-3'<br>5'-TTCTTCAGGACACAGTGGCT-3' |
| HPV 16 E7 | 5'-GCAACCAGA-GACAACTGATCTCTAC-3'<br>5'-GGTCTTCCAAAGTACGAATGTCTACG-3' |
| GAPDH | 5'-AATCCCATCACCATCTTCCAG-3'<br>5'-TCATGAGTCCTTCCACGATACC-3' |
| survivin promoter | 5'-GTTCTTTGAAAGCAGTCGAG-3'<br>5'-GCCAGTTCTTGAATGTAGAG-3' |
| MY09-MY11 | 5'-CGTCCMARRGGAWACTGATC-3'<br>5'-GCMCAGGGWCATAAYAATGG-3'<br>M = A/C, W = A/T, Y = C/T, R = A/G |
| GP5+-GP6+ | 5'-TTTGTTACTGTGGTAGATACTAC-3'<br>5'-GAAAAATAAACTGTAAATCATATTC-3' |

Table 1. Primers used in the articles

*Northern blot hybridization*

RNA samples were run in formaldehyde/ agarose gels, blotted onto Hybond-N+ nylon membranes and hybridized with radioactively labelled survivin and GAPDH probes. Detection of hybridization signal was performed in a Bio-Rad phosphorimager (Personal Molecular Imager FX).

*Study groups, DNA extraction*

Tumour tissues from 81 patients with cancer of the uterine cervix were collected from women who underwent radical hysterectomy with lymphadenectomy. The P3 study group included 122 women with the diagnosis of equivocal cytologic atypia according to the Papanicolaou classification (P3, mild to severe dyskaryosis). The control specimens (peripheral blood samples) were collected from 180 blood donors from the same geographical region as the gynaecologic patients.

The cervical cancer samples were digested by proteinase K, genomic DNA was extracted by phenol–chloroform–isoamyl alcohol (25:24:1), and after ethanol precipitation, pellets were dissolved in TE buffer (10 mM Tris-HCL, pH 8.0, 1.0 mM EDTA). DNA samples of P3 specimens were derived from epithelial cells scraped from the uterine cervix. The genomic DNA of the control population was extracted from white blood cells. After cells were lysed and washed in lysis buffer (0.15 M NH4Cl, 0.01 M KHCO3, 0.01 mM EDTA) three times, DNA was extracted by phenol–chlorophorm–isoamyl alcohol (25:24:1), precipitated with ethanol and resuspended in TE buffer.

*Detection and typing of human papillomavirus (HPV)*

In order to detect HPV, cervical carcinoma samples and P3 samples were analysed by a standard nested PCR with the MY09/MY11 and the GP5+/GP6+ primer set. To determine the HPV type, the product of the nested PCR was then digested by *MseI* and *RsaI* enzymes The fragments were analysed on 3 % agarose gel and stained with ethidium bromide.

*PCR-RFLP (restriction fragment length polymorphism), PCR-SSCP (single strand conformation polymorphism)*

Survivin promoter specific polymerase chain reaction was performed with the primer pair shown in Table 1. The PCR products were digested with the restriction enzyme *Eco*O109I at 37 °C for 16 h. Digestion patterns were analyzed by electrophoresis in a 1.5 %

agarose gel stained with ethidium bromide. We used DNA samples isolated from different cell lines (A549, Caski, HeLa) as controls for PCR-RFLP analysis of the survivin promoter.

After survivin promoter specific PCR, the amplimer was digested with the restriction enzyme *HaeIII* in order to get shorter fragments that are more adequate to single-strand conformation polymorphism (SSCP). The digested PCR porduct diluted in SSCP loading buffer (95 % formamide, 0.05 % bromphenol blue, 0.05 % xylene cyanol) was denatured at 95 °C for 5 min. Samples were then loaded into a 10 % non-denaturing polyacrylamide gel (1 x TBE buffer, 350 V, 4 °C, 4-5 h). Gels were silver stained to visualize DNA bands. For PCR-SSCP, the same control DNA samples were used as for the PCR-RFLP analysis.

*Sequencing*

Survivin promoter specific PCR products were purified with MicroconYM-100 centrifugal filter devices, and survivin promoter variants were sequenced using the BigDye Cycle Sequencing kit with primers used for the PCR. Sequencing was performed using an ABI PRISM 3100 Genetic Analyzer.

*Satistical analysis*

Frequency distribution of the survivin promoter genotypes in the three study groups was analysed by chi-square test. P-value less then 0.05 was considered statistically significant.

## Results and conclusions

*I. Effects of human papillomavirus type 16 oncoproteins on survivin gene expression (I. article)*

Survivin reporter constructs were transfected along with the HPV 16 E6 (pcDNA-16E6), E7 (pcDNA-16E7) and empty expression vector pcDNA in HeLa, MCF-7 and Saos-2 cell lines.

Our results demonstrated that the HPV-16 E6 oncoprotein is able to transactivate the human survivin promoter in human cancer cell lines. This transactivation appeared to be largely dependent on the presence of the cellular tumour suppressor protein p53. It is well established that high-risk HPV E6 proteins induce the proteosome-mediated degradation of p53, which seems to be important in the transformation of the host cells. In Saos-2 cells, which are p53 null, HPV-16 E6 had no transactivating effect on the survivin promoter. On the other hand, we found strong transactivation effect of E6 on the survivin promoter in HeLa and MCF-7 cells, which have functionally active p53 protein. We showed that expression of p53 results in downregulation of the survivin promoter constructs, which we showed to be transactivated by E6. Repression of the human survivin promoter by p53 was also demonstrated by others, although there were some discrepancies in the mechanisms proposed.

A putative p53-binding site is found in the human survivin promoter, and one study found that this site binds p53 and is necessary for the p53-induced repression of the promoter. Other studies reported that repression of the survivin promoter by p53 is not dependent on direct binding of p53 to the promoter. It was suggested that p53 downregulates the survivin promoter by indirect mechanisms, either by altering the chromatin structure through recruiting histone deacetylases or by inducing the expression of the p21/waf1/cip1 gene. Nevertheless, we have defined a role for p53 in the transactivation of the survivin promoter by E6. HeLa cells were transfected with the survivin reporter construct pLuc-230c, along with empty vector, expression vectors containing wild-type or different deletion mutants of 16E6. Two deletion mutants (16E6Δ106-110 and 16E6Δ111-115), which lost p53 degradation activity but retained the ability to transactivate the adenovirus (Ad) E2 promoter, had no or very low transactivating effect on the survivin promoter. Conversely, a mutant (16E6Δ128-132) that had showed almost wild-type p53 degradation, but very low AdE2 transactivation, retained near wild-type transactivation effect on the survivin promoter. These results further suggest

that the ability of E6 to bind p53 and induce its degradation has a major role in the transactivation of the human survivin promoter.

We used HEF cell, to study the effects of the HPV oncogenes on the transcription of the endogenous survivin gene. Control HEF cells (HEF-LXSN) had low survivin mRNA levels, while expression of HPV-16 E6 and/ or E7 (HEF-16E6, -16E7, -16E6E7) by retroviral transduction was found to induce endogenous survivin transcription. This is in accordance with the results of the transient expression assays where we found E6 to transactivate the human survivin promoter. Although HPV-16 E7 did not transactivate the survivin promoter in transient expression assays, it induced endogenous survivin mRNA levels in HEF cells. Consistent with our findings, a previous study, using cDNA expression arrays also found that HPV-16 E6/E7 is able to induce survivin expression in human keratinocytes.

As the expression of survivin is strictly regulated by the cell cycle and the HPV oncoproteins have the ability to modify the cell cycle, we studied whether the effect of the HPV oncogenes on the expression of the survivin gene is dependent on the cell cycle. In HeLa cells, where cells were co-transfected by the survivin reporter construct pLuc-230c along with vector control or HPV-16 E6 expression vector, after synchronization of the cell cycle, the transactivation effect of E6 on the human survivin promoter was independent of the cell cycle. In accordance with this, we found that the induction of the endogenous survivin mRNA by HPV-16 E6/E7 in HEF cells was also independent of the cell cycle. These results indicate that the HPV-16 E6 oncoprotein does not activate survivin transcription through modulation of the cell cycle.

High-risk HPV oncoproteins have effects on apoptosis. HPV-16 E6 tends to inhibit apoptosis in the natural host cells of the virus (primary keratinocytes), when apoptosis is induced by relatively physiological stimuli such as differentiation or Fas ligand. The precise mechanism of the anti-apoptotic function of E6 is not known, but the binding and inhibition of pro-apoptotic proteins (p53, Bax and Bak) are thought to play a role. As p53 downregulates survivin expression, and survivin is an anti-apoptotic protein, it was suggested that p53 induces apoptosis partially through inhibiting survivin transcription. Furthermore, it can be speculated that the p53-mediated transcriptional transactivation of the survivin gene by HPV-16 E6 has a role in the anti-apoptotic function of this oncoprotein.

*II. Survivin promoter polymorphism and cervical carcinogenesis (II. article)*

Control of cell proliferation and apoptosis has a critical role in normal development and cellular homeostasis. Abnormal expression of regulator proteins of apoptosis may lead to dysfunction of the cell, and uncontrolled proliferation. Survivin up-regulation in a number of cancers suggests that apoptosis related genes play important roles in tumour formation and/or progression.

Several studies revealed the importance of survivin over-expression in cancer of the uterine cervix. The over-expression was observed not only in invasive cancer but even before tumour development.

HPVs have long been associated with carcinoma of the uterine cervix. The viral oncoproteins E6 and E7 facilitate the formation of the tumour by influencing the normal process of the cell cycle. Possible correlation between the expression of survivin and high-risk HPV infection was observed in cervical cancer.

The molecular and cellular mechanisms that explain survivin deregulation in cancer has been intensely investigated. Several mechanisms were suggested for the aberrant expression of survivin. These include the amplification of the survivin locus on 17q25 through 17q gain in neuroblastoma[20], the demethylation of survivin exon1 in ovarian cancer, the transcriptional repression of survivin by p53, and the deregulation of the Wnt/TCF signalling pathway. The survivin promoter is largely inactive in normal cell types but is functional in tumour cells. The promoter contains several cell cycle dependent elements (CDE) and cell cycle homology regions (CHR), which are characteristic of G2/M expressed genes. These elements are responsible for the cell cycle dependent expression of the survivin gene. A nucleotide polymorphism (G/C) was recently identified at the –31 nt relative to the ATG start codon. These survivin promoter variants have different transcriptional activities in cancer cell lines. Since the polymorphism is located in the CDE/CHR repressor binding site, it may affect the binding of elements, which regulate cell cycle dependent transcription of the survivin gene, thereby may have a role in the deregulation of survivin in tumours.

In order to determine the relationship between the polymorphism and the development of HPV associated cervical carcinoma, we studied the genotype distribution and allele frequencies of the survivin promoter variants in cervical cancer patients, a P3 (equivocal cytological atypia) population and control group.

Using Restriction Fragment Length Polymorphism (RFLP) after survivin promoter specific PCR, we could determine the nucleotide variants (G/C) at the polymorphic site within the survivin promoter region (at the –31 nt relative to the ATG start codon). In addition to RFLP,

PCR-based single strand conformation polymorphism (SSCP) analysis was used to detect the same sequence variants. On the basis of the SSCP pattern of the control cell lines, we determined the polymorphism. The two tests gave identical results in all cases.

Representative cases of each variant were sequenced as well and the sequences were in complete correlation with the RFLP and SSCP patterns.

There were no significant differences in genotype frequencies between the studied populations ($p = 0.4$). Comparing the genotype distribution of the control group separately with the cancer cases ($p = 0.37$) and with the P3 population ($p = 0.5$), we did not find any significant differences. Similarly, no significant differences in the survivin promoter genotype frequencies were found between the control group and HPV positive P3 and cervical cancer cases ($p = 0.7$) and between the cervical cancer and tumour-free (control and P3) cases ($p = 0.27$).

Since HPV 16 is the most prevalent HPV type in cervical cancer, we also tested the survivin promoter polymorphism in the HPV 16 positive P3 and cervical cancer populations versus the control population. We did not find significant difference in the proportion of genotypes in HPV 16 positive patients compared to the control group ($p = 0,74$).

We calculated the G and C allele frequencies in the study populations (control, P3 and cervical cancer samples) and we did not detect significant difference ($p = 0.99$). The genotypes distribution calculated on the basis of allele frequencies corresponded to the Hardy-Weinberg equilibrium.

We could not find any statistically significant differences in the genotype distributions of the survivin promoter variants among our study groups. This finding suggests that the survivin promoter polymorphism may not represent an increased risk for the development of cervical cancer, at least in our study population. Further studies would be needed to see whether survivin polymorphism has a role in cervical carcinogenesis in other geographical regions.

**Summary**

1. HPV 16 E6 oncoprotein is able to transactivate the human survivin promoter. This transactivation appeared to be largely dependent on the presence of the cellular tumour suppressor protein p53. Experiments with HPV 16 E6 mutant also indicated that transactivation of the survivin promoter by E6 is largely p53-dependent.

2. HPV 16 oncoprotein E7 had no direct effect on the promoter of the survivin gene.

3. HPV 16 E6 and/or E7 induced endogenous survivin transcription in human embryonic fibroblast cells. The effect of E6 and E7 on the endogenous survivin mRNA level and the transactivation effect of E6 on the survivin promoter are independent of the cell cycle.

4. There were no statistically significant differences in the genotype distributions of the genetic polymorphism located in the CDE/CHR repressor elements of the human survivin promoter (at the –31 nt relative to the ATG start codon). We could not find and correlation considering the polymorphism in connection with HPV infection. This survivin promoter polymorphism may not represent an increased risk for the development of cervical cancer, at least in our study population.

**Publication**

**This thesis is based on the following publications:**

I **Ágnes A. Borbély**, Melinda Murvai, József Kónya, Zoltán Beck, Lajos Gergely, Fengzhi Li, and György Veress
Effects of human papillomavirus type 16 oncoproteins on survivin gene expression.
J. Gen. Virol., Feb 2006; 87: 287- 94. IF: 3,221

II **Ágnes A Borbély**, Melinda Murvai, Krisztina Szarka, József Kónya, Lajos Gergely, Zoltán Hernádi, and György Veress
Survivin promoter polymorphism and cervical carcinogenesis.
J. Clin. Pathol., May 2006; (online) IF: 2,619

**Other publications:**

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., Dec 2004; 149: 2379-2392. IF: 1,841

**Presentations and posters**

**Ágnes Anikó Borbély**, Krisztina Szarka, György Veress, Melinda Murvai, Lajos Gergely: Effect of the human papillomavirus type 16 E6 variants on the isoforms of the p53 protein
Jubilee Meeting of the Hungarian Society for Microbiology, October 10-12, 2001, Balatonfüred

**Ágnes Anikó Borbély**, Melinda Murvai, György Veress, Lajos Gergely, Krisztina Szarka: Biological effects of the intratypic variants of the human papillomavirus type 16 (HPV16) E6 oncoprotein
20th International Papillomavirus Conference, October 4-9, 2002, Paris

**Ágnes Anikó Borbély**, Melinda Murvai, Lajos Gergely, György Veress:
Effect of the oncoproteins of human papillomavirus (HPV) type 16 on the transcriptional activity of the survivin gene
14th International Congress of the Hungarian Society for Microbiology, October 9-11, 2003, Balatonfüred

**Ágnes Anikó Borbély**, Melinda Murvai, Lajos Gergely, György Veress:
Effect of the human papillomavirus 16 (HPV) E6 and E7 oncoproteins on survivin gene expression
Annual Meeting of the Hungarian Society for Microbiology and X. Fermentation Kollokvium, October 7-9, 2004, Keszthely

**Ágnes Anikó Borbély**, Melinda Murvai, Lajos Gergely, György Veress:
Relationship between survivin promoter polymorphism and human papillomavirus associated cervical carcinoma
A Magyar Mikrobiológiai Társaság 2005. évi Nagygyűlése és a 1st Central European Forum for Microbiology (CEFORM), 2005. október 26-28., Keszthely