The role of human papillomavirus in the penile cancer

PhD Thesis

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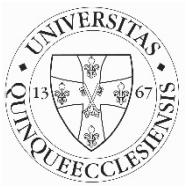

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1. Introduction

Penile cancers are one of the rare forms of oncological diseases, as in developed countries, their prevalence is less than 1%. Epidemiological studies carried out among patients with penile tumour revealed a total of 6 risk factors, which, if present, significantly increase the chance of developing cancer compared to the background population. These factors are: phimosis, smoking, chronic infection (balanoposthitis, balanitis xerotica obliterans), the presence of viral papillae (HPV-infection), sporalene and UV-A phototherapy, promiscuity.

Epidemiological studies also suggested the role of oncogen HPV-types as a pathogenic cause of penile tumours. Currently available literature data explain HPV-induced tumours with the integration of virus into the epithelial cells, and its genetic manipulation of the host DNA. A 2009 review published by Backes and co writers showed that around 40% of patients with penile cancer had also been affected by HPV, among them subtype 16 was the most prevalent from. Another interesting fact is that HPV-infection is much more frequently associated with certain types of penile cancers (e.g. basaloid, condylomatosus 76%), than other cancerous manifestations, (e.g. verrucosus carcinoma 24,5%).

Clinical course and prognosis of patients with penile cancer is unequivocally determined by the lymphatic node status. 5-year survival rate of pathologically negative lymph nodes is 75-93%, while with the involvement of pathologically verified lymph nodes in the pelvis minor, the 5-year survival rate is only 20-34%.

The question is whether the HPV involvement of HPVs in the affected primary tumor, a progressive process, clinically positive, and possibly the removed sentinel lymph node involvement of cloned, is related to HPV involvement, whether the presence of the same subtypes, oncogenic HPV types can be detected in both tissue environments . If so, do these patients show a difference in the clinical course of the cancerous disease compared to the control population.

Extensive research and data collection is only sporadic among men, while the urology society's directives deal with very little human papillomavirus. Like the rest of the world, hungarian urologists has not shown any particular interest in HPV problematic.

We have attempted to overcome this lack of interest in our research in which we investigated the presence of human papillomavirus in the primary tumor and in the lymphatic gland, we sought biological markers that correlated with the progression of penile tumors and the

protective role of toll-like receptor 4 (TLR4) in penile tumors. Additionally, we wanted to summarize the knowledge gained from HPV and penile tumors.

Although HPV's role in cancer is considerably lower than in women, research can be of benefit to this: vaccinations may also have a preventative role in penile cancer cases, and HPV's prognostic role may also be apparent.

2. Objectives

• Finding the frequency of HPV infection in penile cancer, and typing HPV to determine the most common subtype in penile tumors.

• Investigating the protective role of TLR4 in persistent HPV infection, the nuclear integration of the virus and thus the development of penile tumors.

• Investigate the relationship between HPV positivity and TLR4, $p16^{INK4a}$, and p53 tissue expression.

• Proofing or abandoning the development of viral and non-viral infections of the penile tumors.

• Markers related to the progression of penile tumors such as EZH2, MMP12, mTOR, RARRES1 and E-cadherin and vimentin.

3. Patients and methods

Between 2002 and 2012, during operations of malignant penile cancers in the Department of Urology, University of Pécs, tissue samples were taken from both the primer tumour and the regional lymph nodes. Samples were forwarded to histopathological processing, where tissues were embedded in paraffin. The collection and processing of tissue samples was carried out with the permission of the PTE ÁOK Ethics Committee (Ethical Approval Number: 4828).

Table 1. Summary of virological tests and clinical manifestations (i.e. oncology status, surgical interventions)

No. of patients	TNM	Surgery	Age (year)
1	pT1pN0GII	excision	61
2	pT1pN0MxGI	partial amputation	81
3	pT3pN0MxGII-III	amputation	53
4	pT1pN0M0GI	excision	87
5	pT2N2MxGI	partial amputation	82
6	pT1pN0GI	excision	56
7	pT4pN2MxGIII	emasculinisation	44
8	pT1pN0MxGI	excision	54
9	pT3pN2MxGII-III	amputation	53
10	pT1pN2MxGI-II	excision	79
11	pT1pN0GIII-IV	amputation	63
12	pT1pN3MxGIV	excision	56
13	pT2pN2MxGII-III	amputation	50
14	pT4pN2GIII-IV	emasculinisation	52
15	pT3pN1MxGIII-IV	emasculinisation	52
16	pT2pN0MxGII-III	partial amputation	78
17	pT1bpN0MxGIII-IV	excision	60
18	pT1apN0MxGI-II	partial amputation	74
19	pT1pN0MxGI	excision	73
20	pT1CISpN0MxGIII	excision	61
21	pT1bpN3MxGIII-IV	amputation	53
22	pT1pN0MxGII	excision	72
23	pT1pN0MxGII	excision	59
24	pT2pN0MxGII	amputation	44
25	pT2pN0MxGII-III	amputation	60
26	pTapN0MxGI	excision	59
27	pT2pN0MxGI-II	amputation	57
28	pT1pN0MxGII	excision	46
29	pT3pN3MxGI	amputation	78
30	pT2pN3M1GIII-IV	amputation	59
31	pT3pN2MxGIII-IV	amputation	61
32	pT2pN0MxGIII	amputation	48
33	pT3pN2MxGIV	amputation	71
34	pT3pN2MxGII	amputation	56
35	pT2pN0GII	excision	50

3.1 HPV analysis

From paraffin blocks containing tumor tissues 10 μm sections were dewaxed, the tissues were disintegrated using a TissueLyser (Qiagen, Biomarker Ltd., Budapest, Hungary) and the cells were digested with Proteinase-K. DNA was extracted using QIAamp DNA FFPE Tissue Kit (Qiagen, Biomarker Ltd., Budapest, Hungary) according to the manufacturer's recommendations. HPV DNA was detected by virus-specific TaqMan PCR (DIAGON Ltd., Budapest, Hungary). The measurements were performed on a LineGene 9600 real time PCR, where the presence of the viral nucleic acid HPV was confirmed by detecting the fluorescence signal at 530 nm.

3.2 Tissue microarray, TMA

During the study, carcinoma-containing paraffin blocks were used to produce tissue microarray (TMA). During the review of the hematoxylin-eosin painted tumor blocks, we highlighted the sampling location. Subsequently, in the tissue area embedded in paraffin, Manual Tissue Arrayer (MTA1, Beecher Instruments, Inc., Sun Prairie, USA) was used to apply 0.6 mm diameter tissue rolls to the designated area. Several (3-5) samples were taken from each tumor, but most of the tumors with different morphology or squares. The resulting tissue rolls were embedded in a common paraffin block using the MTA1 device, allowing simultaneous examination of all tumors tested on one section.

3.3 Immunhistochemistry

Paraffin was removed with 4 μm thick sections from TMA using xylene, and the sections were rehydrated in descending ethanol sequences. Subsequently, the antigen digestion was achieved in 10 mM sodium citrate buffer (pH 6.0) or TE buffer (pH 9.0), which was performed in 2100 Retriever (Pick-Cell Laboratories, Amsterdam, The Netherlands). Blocking of endogenous peroxidase activity and non-specific binding sites was performed at 3% hydrogen peroxide containing 1% normal horse serum at room temperature for 10 minutes. Subsequently, the sections were incubated with the primary antibody in a humid chamber at 4 ° C.

The following antibodies were used:

- **rabbit anti-TLR4 antibody** (PA5-23124, Thermo-Fisher Scientific, Budapest, Hungary) in 1: 100 dilution;
- **rabbit anti-mTOR antibody** (PA5-34663, Thermo-Fisher) in 1: 100 dilution;
- **rabbit anti-RARRES1 antibody** (PA5-22310, Thermo Fisher) in 1: 200 dilution;
- **rabbit anti-MMP12 antibody** (NBP1-31225, Novus Biologicals, Littleton, CO, USA), diluted 1: 250.

Thirty minutes of HRP conjugated anti-rabbit secondary antibody (MACH4 Universal HRP-Polymer, Biocare Medical, Concord, USA) was developed for thirty minutes using the AEC substrate and DAB (DAKO Glostrup, Danemark) and the sections were stained with Mayer hematoxylin.

The following antibodies were detected in Leica Bond Max Autostainer:

- **rabbit monoclonal anti-P53 antibody** (SP5, Thermo-Fisher) in 1: 200 dilution;
- **rabbit monoclonal anti-p16 antibody** (R19-D, DB Biotech) in 1: 100 dilution;
- **mouse monoclonal anti-EZH2 antibody** (6A10, Novocastra, Leica) in 1: 200 dilution,
- **mouse monoclonal anti-Vimentin antibody** (V9, Boehringer-Manheim, Mannheim) in 1: 1000 dilution;
- **mouse monoclonal anti-E-cadherin antibody** (NHC-38, DAKO) in 1: 200 dilution.

Finally, a double evaluation of the sections was performed without the knowledge of the clinical data. Evaluation was performed using a Leica LaborluxS microscope using a ProgRes C14 camera placed on a Leitz DMRBE microscope.

3.3.1 Evaluation of immunohistological reactions

The results of TLR4, $P16^{INK4a}$ and p53 immunohistology were evaluated twice without HPV status. Evaluation of EZH2, mTOR, RARRES1, MMP12 was performed without knowledge of tumor progression. Since the proportion of positive cells with these antibodies was over 80%, the percentage was not evaluated as a parameter. The evaluation was based solely on the intensity of the reaction: lacking, weak, medium and strong staining. E-cadherin and vimentin immunohistology were positive in only one or two cases and were not evaluated.

3.3.2 Statistical methods

Correlation between HPV positivity, TLR4, p53 and p16 expression was analyzed with Pearson's Chi square test. The results were considered significant if p-value was under 0.05. All calculations were performed with SPSS (IBM Corporation, New York, USA, v24.0).

4. Results

In the study period, the primary tumor was removed in a total of 47 patients and the regional lymph node was removed in 35 cases. Twenty-four patients underwent dynamic statin lymph node determination. Using the DNA preparation method, a sufficient amount of nucleic acid was obtained, which was further used for the detection of viral nucleic acid. In the final studies 35 patients with primary tumor and lymph node samples were tested in parallel. Of course, there was only an opportunity to compare the patients' materials with both primary tumor and regional lymph node (either sting lymph node or lymphadenectomy) and HPV positivity.

In a clinical study of our own patient, in all of the successful 31 patients, 16 patients were able to isolate high malignant HPV DNA from the primary tumor, and in 3 cases we could detect HPV from its regional (inguinal) lymph nodes. In further molecular biological studies and typing processes, in 13 of the 16 high-malignant HPV positive primary tumors, the high sensitivity test method was used to characterize. Types of HPV 16 subtypes in all 10 patients (76.92%) and 3 (23.07%) HPV 51, 82, 59 subtypes were confirmed. In the 3 patients with HPV analysis of both primary and lymph node positive HPV 16 subtypes were positive.

In the clinical and pathological studies associated with molecular biology, 47% pTa-pT1 and 53% pT2-pT4 localized HPV positive cases were confirmed. The clinical study showed that the 3 patients with both primary and regional lymph nodes were positive, primary tumor localized (pT3-pT4) or pelvic lymph node positivity. All three patients had extensive primary tumor removal (emasculinisation), available staging or pathological results, adjuvant chemotherapy would have been necessary due to rapid progression of the process, however, due to poor compliance, no further oncology treatment was performed.

4.1. Immunohistological examination of TLR4, P53 and $P16^{INK4a}$ in penile tumors

4.1.1. TLR4

TLR4 showed diffuse expression in 17 penile tumors, while in 14 cases no immune response was found. Only four of the 16 positive HPV positive tumors showed TLR4 positivity, but TLR4 gene expression was found in 13 tumors of 15 HPV negative tumors. Pearson's Chi square test showed significant inverse correlation between HPV positivity and TLR4 immune response ($p = 0.0006$).

4.1.2. p53

Immunohistological examination of p53 showed a medium or strong intensity staining of 16 colorectal carcinomas. Of the 16 HPV positive penile tumors, only 5 tumors showed positive staining with p53 antibody. On the other side, 11 of the 15 HPV negative tumors (73%) were able to detect strong and diffuse nucleus positivity. Pearson's Chi square test showed significant correlation between missing HPV integration and p53 immune response ($p = 0.0191$).

4.1.3. $p16^{INK4a}$

Expressed $p16^{INK4a}$ immune response was observed in cytoplasm of 10 colorectal tumors and tumor nucleus. All $p16^{INK4a}$ positive malignant HPVs were positive. In contrast, HPV negative penile tumors showed no p16INK4a immune response. Pearson's Chi square test showed a significant correlation between HPV positivity and the expression of $p16^{INK4a}$ protein ($p = 0.0002$).

Table 2. Results of virological and immunohistological (TLR4, $p16^{INK4a}$ and p53) studies

	TNM-G	**HPV/ szubtípus**	**TLR4**	**$p16^{ink4a}$**	**p53**
1	pT2,N0,Mx,G3	pos/16	0	1	1
2	pT2,N0,Mx,G2	pos/16	1	1	0
3	pT2,N0,Mx,G3	pos/16	0	1	0
4	pT1,N0,Mx,G2	pos/16	0	0	0
5	pT2,N0,Mx,G2	pos/16	0	0	0
6	pT1,N3,Mx,G3	pos/16	1	1	1
7	pT1,N0,Mx,G1	pos/16	0	1	1
8	pT4,N2,Mx,G3	pos/16	1	1	0
9	pT4,N2,Mx,G3	pos/16	0	0	0
10	pT3,N1,Mx,G3	pos/16	0	0	0
11	pT1,N0,Mx,G3	pos/59	1	1	1
12	cis	pos/51,82	0	1	0
13	pTa,N0,Mx,G1	pos/82	0	0	0
14	pT1,N3,Mx,G3	pos/na	0	1	1
15	pT3,N0,Mx,G3	pos/na	0	0	0
16	pT3,N2,Mx,G3	pos/na	0	1	0
17	pT3,N2,Mx,G3	neg	1	0	1
18	pT2,N3,M1,G3	neg	1	0	0
19	pT1,N0,Mx,G1	neg	1	0	1
20	pT2,N2,Mx,G1	neg	0	0	0
21	pT1,N0,Mx,G1	neg	0	0	1
22	pT3,N2,Mx,G2	neg	1	0	1
23	pT1,N0,Mx,G1	neg	1	0	1
24	pT1,N2,Mx,G2	neg	1	0	1
25	pT1,N0,Mx,G3	neg	1	0	0
26	pT2,N2,Mx,G3	neg	1	0	1
27	pT2,N0,Mx,G2	neg	1	0	1
28	pT1,N0,Mx,G2	neg	1	0	1
29	pT1,N0,Mx,G1	neg	1	0	1
30	pT1,N0,Mx,G2	neg	1	0	0
31	pT3,N2,Mx,G3	neg	1	0	1

4.2 The relationship between HPV positivity and TLR4, $P16^{INK4a}$ and p53

When comparing HPV integration and expression of the three genes, two tumor groups were distinguished. In the HPV negative tumor group, 10 penile tumors were both positive for TLR4 and p53. Coexpression of the two markers was significant ($p = 0.0198$). HPV positive malignant tumors showed positive immune responses to $P16^{INK4a}$ antibody, while in HPV negative cases we could not detect $P16^{INK4a}$ positivity. Not only the detection of HPV, but also the combined expression of the three genes clearly defined the likelihood of different development of the penile tumor.

4.3. Immunohistology of markers associated with tumor progression

A number of tumor progression associated markers were selected from those not yet or rarely seen in penile tumors. The expression of the progression-related genes (EZH2, mTOR and RARRES1) can be seen in Table 3.

Table 3. Results of virological and immunohistological (EZH2, mTOR and RARRES1) studies

Eset	**TNM-G**	**HPV/ szubtípus**	**EZH2**	**mTOR**	**RARRES1**
1	pT2,N0,Mx,G3	pos/16	0	1	1
2	pT2,N0,Mx,G2	pos/16	1	1	0
3	pT2,N0,Mx,G3	pos/16	0	1	0
4	pT1,N0,Mx,G2	pos/16	0	0	0
5	pT2,N0,Mx,G2	pos/16	0	0	0
6	pT1,N3,Mx,G3	pos/16	1	1	1
7	pT1,N0,Mx,G1	pos/16	0	1	1
8	pT4,N2,Mx,G3	pos/16	1	1	0
9	pT4,N2,Mx,G3	pos/16	0	0	0
10	pT3,N1,Mx,G3	pos/16	0	0	0
11	pT1,N0,Mx,G3	pos/59	1	1	1
12	cis	pos/51,82	0	1	0
13	pTa,N0,Mx,G1	pos/82	0	0	0
14	pT1,N3,Mx,G3	pos/na	0	1	1
15	pT3,N0,Mx,G3	pos/na	0	0	0
16	pT3,N2,Mx,G3	pos/na	0	1	0
17	pT3,N2,Mx,G3	neg	1	0	1
18	pT2,N3,M1,G3	neg	1	0	0
19	pT1,N0,Mx,G1	neg	1	0	1
20	pT2,N2,Mx,G1	neg	0	0	0
21	pT1,N0,Mx,G1	neg	0	0	1
22	pT3,N2,Mx,G2	neg	1	0	1
23	pT1,N0,Mx,G1	neg	1	0	1
24	pT1,N2,Mx,G2	neg	1	0	1
25	pT1,N0,Mx,G3	neg	1	0	0
26	pT2,N2,Mx,G3	neg	1	0	1
27	pT2,N0,Mx,G2	neg	1	0	1
28	pT1,N0,Mx,G2	neg	1	0	1
29	pT1,N0,Mx,G1	neg	1	0	1
30	pT1,N0,Mx,G2	neg	1	0	0
31	pT3,N2,Mx,G3	neg	1	0	1

na - not studied; 0 - negative; 1 - positive

4.3.1 EZH2

EZH2 exhibited diffuse expression in 17 penile tumors, and in 14 cases no immune response was found. Of the 9 progressive malignant tumors, 8 were positive for EZH2 (90%) and only 8 for EZH2 positivity were found in 22 non-progressive malignant tumors. It is worth mentioning that only one penile tumor progressed in the 14 negative EHZ2 cases. The progression of EZH2 immune response and penile tumor showed significant correlation with Fisher's Exact Test ($p = 0.021$).

4.3.2 MMP12

Human macrophage metalloproteinase MMP12 was expressed in most penile tumors, only 7 tumors were negative. More differentiated squamous cell cancers were mostly MMP12 negative. The positivity of tumor cells ranged from mild staining to strongly positive cytoplasmic immune response. Of the 24 MMP12 positive cases, 9 malignant tumors progressed (38%), while the 7 negative tumor remission remained. In the stroma of tumors, macrophages appeared in different numbers, showing MMP12 positivity. The statistics showed no correlation between the MMP12 positivity and the progression of penile tumors.

4.3.3 mTOR

Diffuse mTOR positivity was found in 21 colorectal tumors. Positive staining did not correlate with the histological type of tumors, and positive cases include both anaplastic or well differentiated keratinocapsular cortex. Of the 16 HPV positive tumors, 10 mTORs were positive, though not significant but suggesting some association. Of the 21 mTOR positive tumors, 9 progressive 12 showed remission. No significant correlation was found between mTOR positivity and p53 positivity. Meanwhile, there was a significant correlation between mTOR expression and tumor progression ($p = 0.012$)

4.3.4 E-cadherin and vimentin

In our material, the E-cadherin and vimentin antibodies used and used reliably in every day routine were also investigated. Of the 31 colorectal carcinoma, only 5 cases of E-cadherin positivity were observed

4.3.5 RARRES1

RARRES1 positivity was found in 16 colorectal tumors, in 7 cases a strong immune response was observed in stromate infiltrating immune cells. RARRES1 positivity did not correlate with the histologic type of tumors, and in positive cases both anaplastic or well-differentiated squamous cell carcinomas occurred. Of the 16 HPV positive tumors, only 5 showed positive immune responses, and 11 out of the 15 HPV negative tumors found RARRES1 positivity. Significant correlation was found between the progression of penile tumors and the expression of the RARRES1 gene ($p = 0.045$).

5. Discussion

5.1 The importance of HPV in the development of penile tumors

Both HPV-associated and HPV independent mechanisms may lead to the malignant penile lesion process, distinct from activating already well-known virus oncogenes (E6 and E7), or directly activating tumor suppressor gene inactivation mechanisms. Some studies have shown correlation between the primary tumor HPV virus status and the progression of tumor disease. Studies on HPV positive and negative tumor survival showed significant differences in HPV positive tumors, but contrary studies showed no significant difference between HPV positive and negative tumor survival data.

The HPV involvement of the primary tumor of the penis has been studied and examined by a number of studies as a prognostic factor assessing the division of the profession.

The results of the studies and the viruses present in the tumors encourage a more accurate genotype of our research team. The use of four- and nine-component vaccines of male populations, which were granted in 2008 in the FED, would be indispensable in the light of the above clinical study, as the occurrence of HPV 16/18 types is more common in other non-HPV 16-18 types in comparison. In addition to the additional screening tests, positive samples will first be screened for high risk HPV viruses, and then specifically for the HPV 16/18 genotype in the future.

5.2 Protective effect of TLR4 (toll-like receptor 4) versus persistent HPV infection and nuclear integration of the virus

During our investigations we first discovered the significant inverse correlation between HPV genomic integration and TLR4 ($p = 0.003$). Our results raise the protective role of TLR4 against HPV infection. The immune system of the body plays a significant role in the prevention of viral infections. A total of 11 similarly constructed transmembrane signaling receptors TLR are known. If the TLR recognizes a foreign antigen, such as a virus, activates the NF-kB signal system and leads to the expression of inflammatory cytokines and natural immune system proteins. It can be said that contrary to the E6 and E7 virus oncogenes, the TLR4 signal transfer system leads to confirmation of immune reactions.

Perhaps the best way to understand the role of TLR4 in HPV infection is to take into account the fact that, in the vast majority of cases, HPV only temporarily infects the epithelial cells and only a few individuals develop chronic infections. In the case of HPV infection, activation of TLR4 leads to immediate, non-specific natural immune response, adaptive immune response only develops later and slowly. Immediate natural immune response depends on the cell to identify the pathogenic virus as a foreign agent. TLRs are signal recognition receptors that recognize the conserved components of pathogenic microbes and trigger the natural immune response. It is interesting to mention the frequent occurrence of HPV 16 among high risk viruses in relation to TLR4. Among HPV positive cases HPV 16 occurred in 76.92% of tumors, while HPV 51, HPV 52 and HPV 82 were only observed in one case. This difference can be explained by the fact that HPV 16 can in some cases disable the natural immune response by manipulating the TLR4 expression and thus persisting in the infected cell, which is a condition for genomic integration. Investigations have resulted in similar results in the penile tumor, TLR4 expression is a significant inverse association with HPV positivity ($p = 0.0006$). In view of the above, we can say that TLR4 expression plays a significant role in overcoming HPV virus infection.

6. Conclusion

To sum up, we first introduced the difference between HPV infection and TLR4 expression in the penile tumor. Increased TLR4 expression indicates HPV negative tumors, and $p16^{INK4a}$ expression increases in HPV positive male penile cancer. Our data suggest that TLR4 receptor

expression protects the long-term presence of viral infection. HPV infection and its inverse relationship with p53 expression refers to pathogenesis unrelated to HPV. Based on our findings, we conclude that:

- In 76.92% of HPV 16 subtype integration, malignant tumors have a causal role. The use of bivalent and qadrivalent vaccines for male populations obtained in 2008 in FED would be indispensable in the light of this clinical study, as the incidence of HPV 16/18 types is much more common in other non-HPV 16/18 types. In addition to the additional screening tests, positive samples will first be screened for high risk HPV viruses, and then specifically for the HPV 16/18 genotype in the future.
- In routine pathological studies, p16 positivity can be used to raise suspicion of HPV integration.
- First, we found that epithelial cells expressing the TLR4 receptor are more resistant to chronic HPV infection and thus to HPV integration. We want to implement the TLR4 immune response in the routine histological study of penile tumors.
- HPV and p16 positivity are viral pathomechanisms, while TLR4 and p53 expression support a non-viral tumor formation.
- The immunohistological expression of mTOR, EZH2 and RARRES1 shows a significant correlation with the progression of the penile tumors.
- mTOR is expressed in two thirds of the penile tumors (66%), so that mTOR positivity can be used to treat mTOR inhibitors therapeutically

7. Abbreviations

BXO	Balanitis Xerotica Obliterans
CIN	Cervical Intraepithelial Neoplasia
CRP	C-reaktív protein
DSNB	Dynamic Sentinel Lymph Node Biopsy
EZH2	Enhancer of Zeste Homolog 2
FNAB	Fine-Needle Aspiration Biopsy
HPV	Human Papillomavirus
hrHPV	high risk Human Papillomavirus
LCR	Long Control Region
LOH	Loss of Heterozygosity

lrHPV	low risk Human Papillomavirus
MMP12	Macrophage Metalloproteinase 12
mTOR	mechanistic/*mammalian Target of Rapamycin*
ORF	Open Reading Frame
ORI	Viral Origin of Replication
PCNA	Proliferating Cell Nuclear Antigen
PCR	Polymerase Chain Reaction
RARRES1	Retinoic Acid Receptor Responder 1
SCCAg	Squamous Cell Cancer Antigen
TLR4	Toll Like Receptor 4
TMA	Tissue microarray
URR	Upstream Regulatory Region
UTR	Untranslated Region
UVA	Ultra-Violet A
VIN	Vulvar Intraepithelial Neoplasia

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