HUMAN PAPILLOMAVIRUS-MEDIATED TRANSFORMATION OF THE ANOGENITAL TRACT

Renske D.M. Steenbergen, Jillian de Wilde, Saskia M. Wilting, Antoinette A.T.P. Brink, Peter J.F. Snijders and **Chris J.L.M. Meijer**
Department. Of Pathology, VU University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.

Keywords : Lesions, Cervix, Cancer, Pathologist

Contents

Summary

Infection with high-risk human papillomavirus (HR-HPV) has been associated with intraepithelial neoplasia and carcinomas at various sites of the anogenital tract, including the cervix, vulva, vagina, penis and anus. Although HR-HPV is a necessary cause for cervical cancer, the majority of anal cancers and a subset of cancers at other genital sites, additional (epi)genetic events are required for malignant transformation. HPV-mediated transformation of human epithelial cells has been recognized as a multistep process resulting from deregulated transcription of the viral oncogenes E6 and E7 in the proliferating cells. Interference of E6 and E7 with cell cycle regulators induces genetic instability which drives the continuous selection of oncogenic alterations providing cells with a malignant phenotype. Early genetic events during cervical carcinogenesis associated with immortalization, include deletions at chromosomes 3p, 6 and 10p, whereas amongst others gain of chromosome 3q, loss of chromosome11, and epigenetic alterations such as inactivation of the TSLC1 tumor suppressor gene represent later events associated with tumor invasion.

1. HPV in anogenital cancers

Different areas of the lower anogenital tract share common risk factors for cancer development, such as sexual behaviour, exposure to HPV and smoking. For cervical cancer infection with HR-HPV has been recognized as a necessary cause. A causative role of HPV is also suggested for the majority of anal cancers as well as for a subset of vulvar, vaginal and penile cancers. Besides the anogenital cancers, a subset of head and neck cancers have been associated with HR-HPV infection. The causal relationship between HR-HPV infection and cervical cancer has become evident from epidemiological and functional studies, and hr-HPV has been detected in up to 99.7% of cervical squamous cell carcinomas (SCCs) and 94% to 100% of cervical adeno- and adenosquamous carcinomas.

In anal cancer the HPV detection rates range from 70% to 100%, dependent on gender, localization, sexual orientation and HIV positivity. Although the overall hr-HPV frequency is markedly lower in vulvar carcinomas, specific histological subtypes of vulvar carcinomas, i.e. basaloid and warty carcinomas, display relatively high HPV prevalence rates of 75-100%. In contrast, HPV is only detected in <= 23% of keratinizing carcinomas of the vulva. In vaginal and penile carcinomas the HPV prevalence is about 60% and 30-42%, respectively. As in vulvar carcinomas, penile carcinomas with basaloid features and those of the warty subtype display the strongest association with HPV.

The detection of HPV in only a subset of vulvar, vaginal and penile cancers points to multiple pathways of carcinogenesis for these sites. A heterogeneous etiology of vulvar cancers is supported by the observation of distinct genetic alterations in HPV- positive versus HPV-negative counterparts. Recent genetic analysis of head-and-neck cancers also revealed distinct patterns of genetic alterations in tumours with hr-HPV oncoprotein activity, compared to those without. Evidence for penile carcinomas being etiologically heterogeneous is supported by a study of Ferreux and coworkers in 2003 showing at least three alternative carcinogenic routes based on different mechanisms of interference with the p16INK4a/cyclin D/Rb pathway, one of which could be attributed to hr-HPV E7 oncoprotein activity.

Although HPV infections at different sites of the anogenital tract are common in the general population, a large difference in carcinoma frequency is found at the different sites. Cervical cancer is the second most common cancer amongst women world wide, with a mean age standardized incidence rate varying from 11.3 per 100.000 women in more developed countries to 18.7 per 100.000 women in less developed countries, and a peak incidence of 44.3 per 100,000 women in Middle Africa. The incidence of anal cancer among men having sex with men is similar to that for cervical cancer (35 per 100.000 men) and is even higher in HIV infected men. On the contrary, vaginal, vulvar and penile cancers are very rare, with incidence rates of about one per 100.000 per year. This suggests that the risk of progression is site dependent. A possible explanation for the relatively high incidence of cervical and anal cancer could be the presence of a transformation zone in both the cervix and the anus, which is suggested to be more susceptible to HPV-mediated transformation.

To date considerable data has been collected on the role of HPV in cervical carcinogenesis Although likely, extrapolation to HPV infections at other anogential sites needs further confirmation.

2. HPV and cervical cancer development

Cervical cancer evolves from preexisting noninvasive premalignant lesions, referred to as cervical intraepithelial neoplasias (CINs) or squamous intraepithelial lesions (SILs). These premalignant lesions are classified histologically on the basis of progressive atypia of epithelial cells: CIN I corresponds to mild dysplasia, CIN II to moderate dysplasia and CIN III to both severe dysplasia and carcinoma in situ. CIN I is also classified as low-grade SIL (LSIL) and CIN II/III as high-grade SIL (HSIL). It is still a matter of debate whether cervical cancer generally develops from infected normal cervical epithelium via a sequence of well recognizable CIN I-CIN II-CIN III lesions, or directly via a rapidly induced CIN III lesion. It is of note that CIN I and II lesions often display a viral oncoprotein expression pattern suggestive of a productive viral infection (see below), whereas CIN III lesions exhibit an expression pattern indicative of HPV-induced transformation. Moreover, there are indications that some CIN III lesions may develop rather fast (within 2 years following normal cytology), whereas it would take another 10 to 12 years for most CIN III lesions to develop into invasive cervical carcinoma. This suggests that CIN III represents a heterogeneous disease of which only the advanced stages are likely to have invasive potential and is supported by studies on molecular markers

HPV infections are very common in young women and frequently resolve spontaneously. The life-time risk to ever contract HPV is estimated to be 80%. Despite relatively high HPV prevalence, the ultimate development of cervical cancer is a rare event occurring after a long period of viral persistence. The majority of infected women appear to clear the virus by an effective immune response. Clearance of an high-risk HPV infection has been linked to cytological regression. On the other hand, a persistent HR-HPV infection appears a prerequisite for clinical progression and the development of CIN III and cervical cancer.

3. HPV-mediated transformation: additive events

The long time period between hr-HPV infection and cervical cancer appearance as well as the low frequency of progression of hr-HPV associated premalignant lesions to invasive cancer reflect the multi-step nature of HPV-induced cervical cancer. For the latter, additional genetic and epigenetic events are required. Recent *in vivo* and *in vitro* data have provided more insight into the steps that may contribute to malignant transformation following HPV infection. These steps include deregulated expression of the viral oncogenes E6 and E7, leading to genetic instability and alterations in regulatory host cell genes by which HPV-transformed cells gain an immortal phenotype and subsequent invasive growth properties.

4. Deregulation of E6 and E7 transcription

Active viral replication and virion production is specifically seen in low-grade CIN lesions and benign wart-like lesions, such as condyloma acuminata. In these lesions the

viral genome is maintained as monomeric episomes in basal cell nuclei, and vegetative DNA amplification occurs only in squamous epithelia undergoing terminal differentiation. Usually, low levels of viral mRNA can be detected in the infected basal cells, but viral transcription, including that of the E6 and E7 genes, is markedly increased in the differentiated layers. Other than providing the E1 and E2 proteins necessary for viral DNA replication, HPVs rely entirely on the host cell DNA replication machinery for viral DNA synthesis. By retrovirus-mediated gene transfer it has been demonstrated that HPV E7 alone is necessary and sufficient to induce cellular DNA replication in a differentiation-dependent manner in organotypic raft cultures of primary human keratinocytes. Reactivation of cellular DNA replication has also been shown in the differentiating spinous cells of condylomata and low grade cervical intraepithelial neoplasias. The fact that differentiated cells have already lost the ability to divide explains why expression of E6 and E7 in differentiated layers does not result in cellular transformation. However, when oncogenes become active in the dividing basal cells, as seen in high grade lesions, interference of hr-HPV E6 and E7 with cell cycle regulators can result in transformation.

An increase in E6/E7 expression in proliferating basal-like cells might be due to integration of the viral DNA in the host cell genome, which is observed in most invasive cancers and a subset of high-grade lesions. Conversely, viral integration may be the result of a genetically unstable environment created by deregulated E6/E7 expression in (para)basal cells. HPV integration has been suggested to promote carcinogenesis by a targeted deregulation of critical cellular genes by means of insertional mutagenesis. However, no firm evidence for such an event has yet been found and HPV integration sites appear randomly distributed over the whole genome with a preference for fragile sites. Nevertheless, integration of hr-HPV genomes and the expression of integrate-derived papillomavirus oncogene transcripts (iPOTs) has emerged as a strong progression factor. In vitro studies, using epithelial raft cultures, have shown that altered histone deactelyation may contribute to deregulated E6/E7 expression in proliferating cells.

In summary, it seems that uncontrolled E6/E7 expression in proliferating basal and parabasal epithelial cells is a phenomenon that distinguishes the process of cell transformation from productive viral infection.

5. E6 and E7, the viral oncogenes

Both viral genes E6 and E7 are needed for induction as well as maintenance of a transformed phenotype, particularly by interference with cell cycle control and apoptosis.

High-risk HPV E6 complexes via a cellular protein, E6-associating protein (E6-AP), with the tumor suppressor gene product p53, resulting in rapid ubiquitin-dependent proteolytic degradation of p53. E6-mediated interference with p53 function together with inactivation of the pro-apoptotic protein Bak by E6 prevent cells from undergoing apoptosis, resulting in a state of genetic instability and enhancing the risk of malignant conversion. Over the past years a growing list of other proteins has been identified

which may contribute to the transforming activity. Another interesting p53-independent target of E6 is telomerase.

The E7 gene product of high-risk HPV is the second major transforming protein. HR-HPV E7 interacts with the retinoblastoma tumor suppressor gene product, pRb, and its family members, p107 and p130, thereby interfering with their control on the G1/S transition of the cell cycle. Inactivation of Rb by HR-HPV E7 can be identified by a permanent upregulation of its upstream inhibitor p16 ^{INK4A}. Since p16 ^{INK4A} expression is regulated by an Rb-dependent negative feed-back loop, continuous inactivation of Rb by hr-HPV E7 results in increased p16 ^{INK4A} levels as can be detected in HPV-infected CIN lesions and cervical carcinomas. Hence, the detection of increased p16 ^{INK4A} expression in liquid based cytology specimens may provide a promising marker for the detection of HPV-induced dysplasia with deregulated E7 expression.

In addition to the *Rb*-family members, E7 has been shown to interact with other host cell factors. It still remains to be determined whether and by what mechanism these interactions relate to E7-mediated transformation.

-
-
-

Bibliography

Arias-Pulido H, Joste N, Wheeler CM. Loss of heterozygosity on chromosome 6 in HPV-16 positive cervical carcinomas carrying the DRB1*1501-DQB1*0602 haplotype. Genes Chromosomes.Cancer 2004; 40:277-284.[This study demonstrates the presence of allele losses at chromosome 6 in cervical carcinomas]

Baseman JG, Koutsky LA. the peidemiology of human papillomavirus infections. J Clin Virol 2005;32S:S16-24 [This demonstrates that the life-time risk to ever contract HPV is estimated to be 80%]

Bosch FX, de Sanjose S. Chapter 1: Human papillomavirus and cervical cancer--burden and assessment of causality. J.Natl.Cancer Inst.Monogr 2003;3-13. [This gives a comprehensive overview of epidemiological data demonstrating the causal relation between human papillomavirus infection and cervical cancer]

Braakhuis BJ, Snijders PJ, Keune WJ, Meijer CJ, Ruijter-Schippers HJ, Leemans CR et al. Genetic patterns in head and neck cancers that contain or lack transcriptionally active human papillomavirus. J.Natl.Cancer Inst. 2004; 96:998-1006.[This presents the detection of different chromosomal alterations in head and neck cancers with and without HPV]

Chatterjee A, Pulido HA, Koul S, Beleno N, Perilla A, Posso H et al. Mapping the sites of putative tumor suppressor genes at 6p25 and 6p21.3 in cervical carcinoma: occurrence of allelic deletions in precancerous lesions. Cancer Res. 2001; 61:2119-2123.[This demonstrates the detection of allelic losses on chromosome 6p in cervical (pre)cancerous lesions]

Chen TM, Pecoraro G, Defendi V. Genetic analysis of in vitro progression of human papillomavirus-transfected human cervical cells. Cancer Res. 1993; 53:1167-1171.[This provides evidence for HPV-

induced transformation of epithelial cells resulting from recessive events]

Chen Y, Miller C, Mosher R, Zhao X, Deeds J, Morrissey M et al. Identification of cervical cancer markers by cDNA and tissue microarrays. Cancer Res. 2003; 63:1927-1935.[This presents differentially expressed genes in cervical carcinomas versus controls identified by micro array expression analysis]

Cheng S, Schmidt-Grimminger DC, Murant T, Broker TR, Chow LT. Differentiation-dependent up-regulation of the human papillomavirus E7 gene reactivates cellular DNA replication in suprabasal differentiated keratinocytes. Genes Dev 1995; 9:2335-2349.[This demonstrates the role of HPVE7 in the activation of of the host cell DNA replication machinery]

Cheung AN, Chiu PM, Tsun KL, Khoo US, Leung BS, Ngan HY. Chromosome in situ hybridisation, Ki-67, and telomerase immunocytochemistry in liquid based cervical cytology. J.Clin.Pathol. 2004; 57:721-727.[This presents the detection of cancer markers in liquid based cytology samples]

Chin-Hong PV, Palefsky JM. Natural history and clinical management of anal human papillomavirus disease in men and women infected with human immunodeficiency virus. Clin.Infect.Dis. 2002; 35:1127-1134.[This present the detection of HPV in anal carcinomas]

Chow LT, Broker TR. Papillomavirus DNA replication. Intervirol. 1994; 37:150-158.[This reviews the role of HPV proteins in DNA replication]

Cottage A, Dowen S, Roberts I, Pett M, Coleman N, Stanley M. Early genetic events in HPV immortalised keratinocytes. Genes, Chromosomes and Cancer 2001; 30:72-79.[This presents chromosomal alerations associated with HPV-induced immortalization]

Cubilla AL, Reuter VE, Gregoire L, Ayala G, Ocampos S, Lancaster WD et al. Basaloid squamous cell carcinoma: a distinctive human papilloma virus-related penile neoplasm: a report of 20 cases. Am.J.Surg.Pathol. 1998; 22:755-761.[This presents the detection of HPV in penile cancers]

Daling JR, Madeleine MM, Johnson LG, Schwartz SM, Shera KA, Wurscher MA et al. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. Cancer 2004; 101:270-280.[This demonstrates the detection of HPV in anal carcinomas]

Daling JR, Madeleine MM, Schwartz SM, Shera KA, Carter JJ, McKnight B et al. A population-based study of squamous cell vaginal cancer: HPV and cofactors. Gynecol.Oncol. 2002; 84:263-270. .[This demonstrates the detection of HPV in vaginal cancer]

Davies R, Hicks R, Crook T, Morris J, Vousden K. Human papillomavirus type 16 E7 associates with a histone H1 kinase and with p107 through sequences necessary for transformation. J.Virol. 1993; 67:2521-2528.[This demonstrates the interaction between HPV 16 E7 and p107 and p130]

Demeter LM, Stoler MH, Broker TR, Chow LT. Induction of proliferating cell nuclear antigen in differentiated keratinocytes of human papillomavirus-infected lesions. Hum.Pathol. 1994; 25:343-348.[This demonstrates that HPV is dependent on the host cell machinery for DNA replication]

Duensing S, Munger K. Centrosome abnormalities and genomic instability induced by human papillomavirus oncoproteins. Prog.Cell Cycle Res. 2003; 5:383-391.[This describes the interference of HPV E6 and E7 with cell division]

Dürst M, Dzarlieva-Petrusevka T, Boukamp P, Fusenig NE, Gissmann L. Molecular and cytogenetic analysis of immortalized human primary keratinocytes obtained after transfection with human papillomavirus type 16 DNA. Oncogene 1987; 1:251-256.[This is one of the first publications describing the induction of immortalization by high risk HPV]

Durst M, Glitz D, Schneider A, zur HH. Human papillomavirus type 16 (HPV 16) gene expression and DNA replication in cervical neoplasia: analysis by in situ hybridization. Virology 1992; 189:132-140.[This demonstrates that E6E7 expression is differentiation dependent]

Dyson N, Howley PM, Münger K, Harlow E. The human papillomavirus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 1989; 243:934-940.[This present the interaction between E7 and pRb]

Ferreux E, Lont AP, Horenblas S, Gallee MP, Raaphorst FM, von Knebel DM et al. Evidence for at least three alternative mechanisms targeting the p16INK4A/cyclin D/Rb pathway in penile carcinoma, one of which is mediated by high-risk human papillomavirus. J.Pathol. 2003; 201:109-118.[This demonstrates

the involvement of of HPV in penile cancer development as well as alternative routes of penile carcinogenesis]

Flowers LC, Wistuba II, Scurry J, Muller CY, Ashfaq R, Miller DS et al. Genetic changes during the multistage pathogenesis of human papillomavirus positive and negative vulvar carcinomas. J.Soc.Gynecol.Investig. 1999; 6:213-221.[This presents genetic alterations detected in carcinomas of the vulva

Frisch M, Fenger C, van den Brule AJ, Sorensen P, Meijer CJ, Walboomers JM et al. Variants of squamous cell carcinoma of the anal canal and perianal skin and their relation to human papillomaviruses. Cancer Res. 1999; 59:753-757. .[This demonstrates the detection of HPV in anal carcinomas]

Guo Z, Hu X, Afink G, Ponten F, Wilander E, Ponten J. Comparison of chromosome 3p deletions between cervical precancers synchronous with and without invasive cancer. Int.J.Cancer 2000; 86:518-523.[This presents allelic losses at 3p in CIN lesions and cervical carinomas]

Hampton GM, Penny LA, Baergen RN, Larson A, Brewer C, Liao S et al. Loss of heterozygosity in cervical carcinoma: subchromosomal localization of a putative tumor-suppressor gene to chromosome 11q22-q24. Proc.Natl.Acad.Sci.USA 1994; 91:6953-6957.[This demonstrates the detection of allelic losses at 11q in cervical carcinomas]

Hawley-Nelson P, Vousden KH, Hubbert NL, Lowy DR, Schiller JT. HPV 16 E6 and E7 proteins cooperate to immortalize human foreskin keratinocytes. EMBO J. 1989; 8:3905-3910. .[This describes the induction of immortalization by HPV16 E6 and E7]

Hayflick L. The limited in vitro lifetime of human diploid cell strains. Exp.Cell Res. 1965; 37:614.{This provides first description of somatic cells having a limited lifespan]

Heselmeyer K, Macville M, Schrock E, Blegen H, Hellstrom A-C, Shah K et al. Advanced-stage cervical carcinomas are defined by a recurrent pattern of chromosomal aberrations revealing high genetic instability and a consistent gain of chromosome arm 3q. Genes, Chromosomes and Cancer 1997; 19:233-240.[This describes CGH analysis of cervical carcinomas and the detection of gains at 3q]

Heselmeyer-Haddad K, Janz V, Castle PE, Chaudhri N, White N, Wilber K et al. Detection of genomic amplification of the human telomerase gene (TERC) in cytologic specimens as a genetic test for the diagnosis of cervical dysplasia. Am.J.Pathol. 2003; 163:1405-1416.[This presents the detection of hTERC DNA amplification in cervical scrapings]

Ho GYF, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. The New England Journal of Medicine 1998; 338:423-428.[This describes that a persistent high riskHPV infection is a prerequisite for the development of CIN III or invasive cancer]

Huibregtse JM, Scheffner M, Howley PM. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. Molecular & Cellular Biology 1993; 13:775-784.[This presents the identification of the E6 interacting factor E6AP]

IARC Working group. Monographs on the evaluation of carcinogenic risks to humans. Human papillomaviruses. Lyon: IARC Scientific Publication, 1995.[This describes a.o. the prevalence of HPV in penile and vaginal carcinomas]

Jesudasan RA, Rahman RA, Chandrashekharappa S, Evans GA, Srivatsan ES. Deletion and translocation of chromosome 11q13 sequences in cervical carcinoma cell lines. American Journal of Human Genetics 1995; 56:705-715. .[This demonstrates the detection of allelic losses at 11q in cervical carcinomas]

Kersemaekers AM, van de Vijver MJ, Kenter GG, Fleuren GJ. Genetic alterations during the progression of squamous cell carcinomas of the uterine cervix. Genes, Chromosomes and Cancer 1999; 26:346-354.[This presents a.o. losses at 6p in cervical carcinomas and CIN III lesions]

Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PLC et al. Specific association of human telomerase activity with immortal cells and cancer. Science 1994; 266:2011-2015.[This describes the presence of telomerase activity in immortal cells and cancer cells]

Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U et al. Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. Int.J.Cancer 2001;

92:276-284.[This describes the upregulationof p16 expression as a result of HPV E7 expression in dividing cells]

Klaes R, Woerner SM, Ridder R, Wentzensen N, Duerst M, Schneider A et al. Detection of high-risk cervical intraepithelial neoplasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes. Cancer Res. 1999; 59:6132-6136.[This presents the detection of ingrated viral DNA in cervical lesionsusing a PCR-based assay-iPOT]

Klingelhutz AJ, Barber SA, Smith PP, Dyer K, McDougall JK. Restoration of telomeres in human papillomavirus-immortalized human anogenital epithelial cells. Molecular & Cellular Biology 1994; 14:961-969.[This demonstrates that the induction of immortalization in HPV transfected cells is associated with an arrest of telomere shortening]

Klingelhutz AJ, Foster SA, McDougall JK. Telomerase activation by the E6 gene product of human papillomavirus type 16. Nature 1996; 380:79-82.[This presents evidence for HPV 16 E6 being able to activate telomerase]

Koi M, Morita H, Yamada H, Saboh H, Barrett JC, Oshimura M. Normal human chromosome 11 suppresses tumorigenicity of human cervical tumor cell line SiHa. Mol.Carcinog. 1989; 2:12-21.[This demonstrates that chromosom 11 has tumor suppressor activity in cervical cancer cells]

Koopman LA, van der Silk A, Giphart MJ, Fleuren GJ. Human Leukocyte Antigen (HLA) class I gene mutations in cervical cancer. J.Natl.Cancer Inst. 1999; 91:1669-1677.[This describes the detection of mutations in HLA genes in cervical cancers]

Koutsky LA, Holmes KK, Critchlow CW, Stevens CE, Paavonen J, Beckmann AM et al. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. N.Engl.J.Med. 1992; 327:1272-1278.[This presents data showing that it takes 10-12 years for a CIN III lesion to develop into an invasive cancer]

Madeleine MM, Daling JR, Carter JJ, Wipf GC, Schwartz SM, McKnight B et al. Cofactors with human papillomavirus in a population-based study of vulvar cancer. J.Natl.Cancer Inst. 1997; 89:1516-1523. .[This demonstrates the detection of HPV in vulvar cancer]

Meyerson M, Counter CM, Eaton ENg, Ellisen LW, Steiner P, Dickinson Caddle S et al. hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell 1997; 90:785-795.[This describes the identification of hTERT/hEST2]

Munger K, Howley PM. Human papillomavirus immortalization and transformation functions. Virus Research 2002; 89:213-228.[This reviews the transforming capacities of high risk HPV typesand the various hoat cell factors the viral proteins interact with]

Münger K, Phelps WC, Bubb V, Howley PM, Schlegel RM. The E6 and E7 genes of human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes. J.Virol. 1989; 63:4417-4421. .[This describes the induction of transformation by high risk HPV]

Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N.Engl.J.Med. 2003; 348:518-527.[This presents a classification of HPV types in oncogenic/high-risk and non-oncogenic virus types based on epidemiological data]

Narayan G, Arias-Pulido H, Koul S, Vargas H, Zhang FF, Villella J et al. Frequent Promoter Methylation of CDH1, DAPK, RARB, and HIC1 Genes in Carcinoma of Cervix Uteri: Its Relationship to Clinical Outcome. Mol.Cancer 2003; 2:24.[This describes the detection of promoter methylation in cervical carcinomas]

Ngan HY, Cheung AN, Liu SS, Liu KL, Tsao SW. Telomerase assay and HPV 16/18 typing as adjunct to conventional cytological cervical cancer screening. Tumour.Biol. 2002; 23:87-92.[This describes the detection of telomerase in cervical scrapings]

Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, Rozendaal L, Voorhorst FJ, Bezemer PD et al. Cytological regression and clearance of high-risk human papillomavirus in women with an abnormal cervical smear. Lancet 2001; 358:1782-1783.[This presents data showing that clearance of HPV infections is linked to cytological regression]

Nobbenhuis MA, Walboomers JM, Helmerhorst TJ, Rozendaal L, Remmink AJ, Risse EK et al. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. Lancet 1999; 354:20-25. .[This describes that a persistent high riskHPV infection is a prerequisite for the development of CIN III or invasive cancer]

Pirisi L, Yasumoto S, Feller M, Doniger J, DiPaolo JA. Transformation of human fibroblasts and keratinocytes with human papillomavirus type 16 DNA. J.Virol. 1987; 61:1061-1066. .[This is one of the first publications describing the induction of immortalization by high risk HPV]

Poignee M, Backsch C, Beer K, Jansen L, Wagenbach N, Stanbridge EJ et al. Evidence for a putative senescence gene locus within the chromosomal region 10p14-p15. Cancer Res. 2001; 61:7118-7121.[This demonstrates that introduction of chromosome 10 in HPV immortalized cells suppresses immortality]

Rader JS, Gerhard DS, O'Sullivan MJ, Li Y, Li L, Liapis H et al. Cervical intraepithelial neoplasia III shows frequent allelic loss in 3p and 6p. Genes, Chromosomes and Cancer 1998; 22:57-65. .[This presents allelic losses at 3p and 6p in CIN lesions and cervical carinomas]

Rao PH, Arias-Pulido H, Lu XY, Harris CP, Vargas H, Zhang FF et al. Chromosomal amplifications, 3q gain and deletions of 2q33-q37 are the frequent genetic changes in cervical carcinoma. BMC.Cancer 2004; 4:5.[This describes the detection of chromosomal alterations in cervical carcinomas]

Reesink-Peters N, Helder MN, Wisman GB, Knol AJ, Koopmans S, Boezen HM et al. Detection of telomerase, its components, and human papillomavirus in cervical scrapings as a tool for triage in women with cervical dysplasia. J.Clin.Pathol. 2003; 56:31-35.[This demonstrates that the detection of telomerase in scrapings is not sensitive and specific enough to predict cervical cancer]

Rubin MA, Kleter B, Zhou M, Ayala G, Cubilla AL, Quint WG et al. Detection and typing of human papillomavirus DNA in penile carcinoma: evidence for multiple independent pathways of penile carcinogenesis. Am.J.Pathol. 2001; 159:1211-1218. .[This demonstrates the detection of HPV in penile cancer]

Sahebali S, Depuydt CE, Segers K, Moeneclaey LM, Vereecken AJ, Van Marck E et al. P16INK4a as an adjunct marker in liquid-based cervical cytology. Int.J.Cancer 2004; 108:871-876.[This describes the value of p16 as a marker for HPV in liquid based cytology]

Schiffman M, Kjaer SK. Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. J.Natl.Cancer Inst.Monogr 2003;14-19. .[This demonstrates the detection of HPV in anogenital cancer]

Schiffman M, Wheeler CM, Castle PE. Human papillomavirus DNA remains detectable longer than related cervical cytologic abnormalities. J.Infect.Dis. 2002; 186:1169-1172. .[This presents data showing that clearance of HPV infections is linked to cytological regression]

Seagon S, Dürst M. Genetic analysis of an in vitro model system for human papillomavirus type 16-associated tumorigenesis. Cancer Res. 1994; 54:5593-5598.[This presents the genetic basis for malignant progression of an human papillomavirus type 16 immortalized human keratinocyte cell line by somatic cell fusion]

Snijders PJ, van Duin M, Walboomers JMM, Steenbergen RDM, Risse EKJ, Helmerhorst TJM et al. Telomerase activity exclusively in cervical carcinomas and a subset of cervical intraepithelial neoplasia grade III lesions: strong association with elevated messenger RNA levels of its catalytic subunit and high-risk human papillomavirus DNA. Cancer Res. 1998; 58:3812-3818.[This presents the detection of telomerase and elevated hTERT mRNA in 40% of CIN III lesions and 96% of cervical carcinomas]

Soder AJ, Hoare SF, Muir S, Going JJ, Parkinson EK, Keith WN. Amplification, increased dosage and in situ expression of the telomerase RNA gene in human cancer. Oncogene 1997; 14:1013-1021.[This demonstrates amplification and increased expression of hTR in cervical carcinomas]

Steenbergen RD, Kramer D, Braakhuis BJ, Stern PL, Verheijen RH, Meijer CJ et al. TSLC1 gene silencing in cervical cancer cell lines and cervical neoplasia. J.Natl.Cancer Inst. 2004; 96:294-305.[this presents TSLC1 gene silenicng by promoter methylation in cervical neoplasia as well as a functional role of TSLC1 in cervical carcinogenesis]

Steenbergen RD, OudeEngberink VE, Kramer D, Schrijnemakers HF, Verheijen RH, Meijer CJ et al. Down-regulation of GATA-3 expression during human papillomavirus-mediated immortalization and

cervical carcinogenesis. Am.J.Pathol. 2002; 160:1945-1951.[This demonstrates downregulation of the transcription fator GATA-3 in HPV immortalized cells and cervical carcinomas]

Steenbergen RDM, Hermsen MAJA, Walboomers JMM, Meijer GA, Baak JPA, Meijer CJLM et al. Non-random allelic losses at 3p, 11p and 13q during HPV-mediated immortalization and concomitant loss of terminal differentiation of human keratinocytes. Int.J.Cancer 1998; 76:412-417. [This demonstrates the detection of clonal genetic alterations and a loss of differentiation in HPV immortalized keratinocytes]

Steenbergen RDM, Kramer D, Meijer CJLM, Walboomers JMM, Trott DA, Cuthbert AP et al. Telomerase suppression by chromosome 6 in a human papillomavirus type 16-immortalized keratinocyte cell line and in a cervical cancer cell line. J.Natl.Cancer Inst. 2001; 93:865-872.[This provides evidence for chromosome 6 harbouring a telomerase suppressor gene]

Steenbergen RDM, Walboomers JMM, Meijer CJLM, van der Raaij-Helmer, Parker JN, Chow LT et al. Transition of human papillomavirus type 16 and 18 transfected human foreskin keratinocytes towards immortality: Activation of telomerase and allele losses at 3p, 10p, 11q and/or 18q. Oncogene 1996; 13:1249-1257.[This presents the analysis HPV 16 and 18-immortalized keratinocytes showing clonal allelic losses and activation of telomerase]

Stoler MH, Rhodes CR, Whitbeck A, Wolinsky SM, Chow LT, Broker TR. Human papillomavirus type 16 and 18 gene expression in cervical neoplasia. Human Pathology 1992; 23:117-128.[This demonstrates that E6E7 expression is differentiation dependent]

Thomas M, Banks L. Human papillomavirus (HPV) E6 interactions with Bak are conserved amongst E6 proteins from high and low risk HPV types. J.Gen.Virol. 1999; 80 (Pt 6):1513-1517.[This shows inactivation of the pro- apoptotic protein BAK by HPV E6]

Trimble CL, Hildesheim A, Brinton LA, Shah KV, Kurman RJ. Heterogeneous etiology of squamous carcinoma of the vulva. Obstet.Gynecol. 1996; 87:59-64. .[This demonstrates the detection of HPV in vulvar cancer]

van Duin M, Steenbergen RD, De Wilde J, Helmerhorst TJ, Verheijen RH, Risse EK et al. Telomerase activity in high-grade cervical lesions is associated with allelic imbalance at 6Q14-22. Int.J.Cancer 2003; 105:577-582.[This shows that activation of telomerase is associated with allelic imbalance at 6q14-22 in CIN III lesions]

van Muyden RC, ter Harmsel BW, Smedts FM, Hermans J, Kuijpers JC, Raikhlin NT et al. Detection and typing of human papillomavirus in cervical carcinomas in Russian women: a prognostic study. Cancer 1999; 85:2011-2016.[This describes the detection of HPV in cervical adenocarcinomas]

Walboomers JMM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J.Pathol. 1999; 189:12-19.[This demonstrates that (virtually) all cervical carcinomas contain HPV DNA]

Wentzensen N, Vinokurova S, von Knebel DM. Systematic review of genomic integration sites of human papillomavirus genomes in epithelial dysplasia and invasive cancer of the female lower genital tract. Cancer Res. 2004; 64:3878-3884.[This demonstrates that viral integration sites are randomly distributed over the whole genome with a preference for fragile sites]

Widschwendter A, Muller HM, Fiegl H, Ivarsson L, Wiedemair A, Muller-Holzner E et al. DNA methylation in serum and tumors of cervical cancer patients. Clin.Cancer Res. 2004; 10:565-571.[This demonstrates promoter methylation in serum and tumor of cervical carcinoma patients]

Williams GR, Lu QL, Love SB, Talbot IC, Northover JM. Properties of HPV-positive and HPV-negative anal carcinomas. J.Pathol. 1996; 180:378-382. .[This demonstrates the detection of HPV in anal carcinomas]

Wistuba II, Montellano FD, Milchgrub S, Virmani AK, Behrens C, Chen H et al. Deletions of chromosome 3p are frequent and early events in the pathogenesis of uterine cervical carcinoma. Cancer Res. 1997; 57:3154-3158. .[This presents allelic losses at 3p in CIN lesions and cervical carinomas]

Zhang A, Maner S, Betz R, Angstrom T, Stendahl U, Bergman F et al. Genetic alterations in cervical carcinomas: frequent low-level amplifications of oncogenes are associated with human papillomavirus infection. Int.J.Cancer 2002; 101:427-433.[This demonstrates copy number gains of oncogenes putatively involved in cervical carcinogenesis]

Zhao W, Noya F, Chen WY, Townes TM, Chow LT, Broker TR. Trichostatin A up-regulates human papillomavirus type 11 upstream regulatory region-E6 promoter activity in undifferentiated primary human keratinocytes. J.Virol. 1999; 73:5026-5033.[This shows that altered histone deacetylation may contribute to deregulated E6E7 expression in dividing cells]

Zielinski G.D., Snijders PJ, Rozendaal L, Voorhorst FJ, Runsink AP, de Schipper FA et al. High-risk HPV testing in women with borderline and mild dyskaryosis: long-term follow-up data and clinical relevance. J.Pathol. 2001; 195:300-306. .[This describes that a persistent high riskHPV infection is a prerequisite for the development of CIN III or invasive cancer]

Zielinski GD, Snijders PJ, Rozendaal L, Daalmeijer NF, Risse EK, Voorhorst FJ et al. The presence of high-risk HPV combined with specific p53 and p16INK4a expression patterns points to high-risk HPV as the main causative agent for adenocarcinoma in situ and adenocarcinoma of the cervix. J.Pathol. 2003; 201:535-543. .[This describes the detection of HPV in cervical adenocarcinomas]

zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat.Rev.Cancer 2002; 2:342-350.[This is a comprehensive review of the role of HPV in (cervical) cancer development]

Biographical Sketches

Renske D.M. Steenbergen, PhD, received her MSc degree in Biomedical Sciences at the University of Leiden, The Netherlands in 1991. In 1997 she received her PhD degree at the VU university in Amsterdam The Netherlands. In 1999 she was awarded a fellowship of the Royal Netherlands Academy of Sciences, which resulted in a faculty position at the Dept. of Pathology at the VU Medical Center in Amsterdam, the Netherlands.

She is a PI on a number of research project related to HPV-induced transformation using both in vitro model system and clinical material. The research of Renske Steenbergen and colleagues focuses on the elucidation of the multistep process of cervical carcinogenesis aiming at the identification of novel biomarkers and therapeutic targets for cervical cancer.

Jillian de Wilde, received her MSc degree in Biomedical Sciences at the VU University in Amsterdam, The Netherlands, in 2003. From 2002 till present she has been working as a PhD student at the Dept. of Pathology at the VU University Medical Center in Amsterdam, the Netherlands. The research of Jillian de Wilde and co-workers focuses on the identification of the mechanism(s) underlying telomerase/hTERT deregulation during HPV-mediated transformation and cervical carcinogenesis.

Saskia M. Wilting, received her MSc degree in Biomedical Sciences at the University of Leiden, The Netherlands in 2002. From 2003 till present she has been working as a PhD student at the Dept. of Pathology at the VU University Medical Center in Amsterdam, the Netherlands. The research of Saskia Wilting and co-workers focuses on the genetic profiling of human papillomavirus induced cervical carcinomas and precursor lesions, ultimately aiming at the identification of novel biomarkers for the early detection of cervical cancer.

Prof. Peter Snijders received his degree in biology (with main focus on molecular biology) at the Catholic University in Nijmegen in 1987 and his doctoral degree at the VU University in Amsterdam in 1992 (cum laude). From 1992 he is staff member of the department of Pathology, VU University medical center, Amsterdam, and since 2000 head of the unit of Molecular Pathology within this department.

He is PI of a number of studies in the field of HPV in relation to particularly anogenital and head and neck cancers. He was involved in the development of the HPV GP5+/6+ consensus PCR method that is amongst the most widely used HPV PCR assays today. In addition, he was amongst the first to establish a plausible etiological role of mucosal HPV in tonsillar cancer. In current research lines HPV-induced oncogenic progression is investigated using both in vitro models and clinically well-defined patient material, aiming at the identification and characterization of genes involved in this process and candidate markers for risk assessment. Viral and host markers are already being tested in screening and clinical trials for their capability to assess the risk of cervical cancer and high-grade precursor stages, and newly identified markers will be investigated likewise. Part of his work is funded by the Dutch Cancer Society (NKB) and the Netherlands Organization for Health and Development (ZonMW).

He is (co)author of over 120 international (peer reviewed) papers in his area of expertise.

Prof. Chris Meijer is a pathologist who received his medical doctor degree at the state University in Leiden in 1972. During his medical study he worked on experimental models , investigating the cellular and (immuno)histochemical changes in lymph nodes draining skin areas of rodents with contact hypersensitivity and received for this work in 1971 his PhD at the Vrije Universiteit in Amsterdam. He is certified as surgical Pathologist and as a medical immunologist. He worked at the Central laboratory of the Blood transfusion service in Amsterdam (1972-1973) on in vitro models of cell mediated immune reactions and received his training in Pathology at the State University Leiden under the guidance of Prof Th.G van Rijssel and Prof. A.Schaberg(1973-1977). He was staff member and head of the Laboratory for Experimental Pathology at the Dept of Pathology, State University Leiden (1977-1980). He moved to Delft where he became staff member and head of the Laboratory for immunopathology in the SSDZ in Delft (1980-1982). In 1983 he was appointed as Prof of Pathology and became head and director of the Department of pathology at the Vrije Universiteit medical center, Amsterdam. In this position he integrated the results of basic and translational research on cancer and immunology in new diagnostic assays for pathology labs and restructured and implemented three successful research lines in his department. He is in the board of several (Bio)medical Journals and presently member of the research council of the Dutch society for the fight against cancer.(KWF/NKB) He has chaired and served on many committees with focus on research or policy making in oncology, immunology and pathology. His research interests are in the field of oncology pathology and immunology and more specifically concern haematopathology, especially non Hodgkins lymphoma and the relationship between viruses and cancer. Chris Meijer has published more than 730 scientific articles in international peer reviewed journals, is the (co-)inventor of several patents in the field of cervical cancer, including new HPV detection methods and received the Marie Parijs Prize for clinical research (1981), the van Vlissingen prize for excellent research on cutaneous lymphomas (1999)and the Eurogin service award for excellent clinical research in the field of cervical cancer (2003). Since 1986 he is heading a group of scientists working on basic and translational aspects of HPV in cervical cancer. He is currently involved in implementing new preventive strategies for cervical cancer in The Netherlands and developing countries, including prophylactic HPV vaccination and HPV testing as primary tool in cervical screening, using cost-effectiveness calculations based on data sampled in randomised clinical trials of which he was/is the principal investigator.

Antoinette A.T.P. Brink, PhD, received a MSc degree in Medical Biology at the Radboud University of Nijmegen, The Netherlands in 1994. In 2000 she received her PhD at the VU University in Amsterdam, The Netherlands. Between 2000 and 2003 she worked as a postdoc in the laboratory of Molecular Cell Biology at the University of Leiden, The Netherlands, studying human papillomavirus integration. In 2003, she was appointed at the VU University again to work on the development of improved HPV detection methods and as a technical supervisor for molecular diagnostics.