Biomolecular and epidemiological aspects of human papillomavirus induced cervical carcinogenesis

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Chapter 8

Summary

In **CHAPTER 1** an introduction to several aspects of cervical cancer, HPV and their relation with immunology is given. It took almost one-and-a-half centuries from the first mention of a possible relationship between sexual intercourse and cervical cancer and the epidemiological evidence that the sexually transmittable HPV is the unifying risk factor of cervical cancer. The tumour process starts in the cervical transformation zone and develops through several stages to cervical carcinoma. Treatment of premalignant and early stages is often successful, but advanced stages are more difficult to cure. By introducing organised, population-based screening programmes developed countries have effectively reduced cervical carcinoma incidence and mortality rates. Developing countries remain areas where high incidences and prevalence of advanced stages are observed. Approximately 80% of the women worldwide are estimated to experience an HPV infection at least once, but the vast majority is able to clear the infection. HPV and HPV induced lesions are eliminated by the cellular immune system when successfully presented on HLA molecules. Genetically caused HLA aberrations can obviously disturb this process and this is thought to be of great significance in cervical carcinogenesis. This complicates the development of preventive and therapeutic HPV vaccines, the focus of much research. In the mean time a great deal of effort is put into implementing organised screening programmes in less economically developed (high-risk) countries.

The outline of this thesis is presented, which addresses epidemiological, immunogenetic and viral aspects in premalignant and invasive cervical lesions.

In **CHAPTER 2** we determined the prevalence of cytological abnormalities in cervical smears of women attending the first organised screening programme in Suriname and compared the prevalences in four Surinamese ethnicities with different cervical carcinoma incidence. Papanicolaou staining and cytological screening were performed on 807 cervical smears taken from Maroons, Amerindians, Javanese and Hindustani. Cervical cytological abnormalities were detected in 13.4% of the assessable smears, of which 2.6% were moderate and severe dysplasia. The cytological abnormalities varied between the ethnicities. In the smears of the Maroons significantly more cytological abnormalities were detected. We observed a high prevalence of moderate and severe dysplasia in all ethnicities, which correlates with the high cervical carcinoma incidence in Suriname. A significantly higher prevalence of mild abnormalities in the Maroons was seen, which did not reflect the relatively low cervical cancer incidence in this ethnicity. However, this can feasibly be explained by the possibility that these women have a different sexual lifestyle, leading to a higher prevalence of transient HPV infection.

Incidence rates of cervical cancer and its precursors vary considerably and are influenced by endogenous and exogenous factors. In **CHAPTER 3** we compared cytological abnormality incidence rates from a high-risk population in the original high-risk area with those

of women from this high-risk population who have immigrated to a low-risk area to give insight in the significance of these factors. Smears collected from Surinamese women attending the Surinamese screening programme and smears collected from immigrant Surinamese women attending the Dutch screening programme were cytologically analysed using the Dutch microscopical coding system KOPAC. The age-adjusted odds of having dysplasia were higher for Surinamese women living in Suriname versus Surinamese immigrant women and increased with increasing stage of atypical changes. We concluded that fewer cases with dysplasia are present in a high-risk population that has immigrated to a low-risk area for cervical cancer than in the high-risk population continuously living in a high-risk area. This finding emphasises the importance of environmental factors.

Loss at chromosome 6p21.3, the human leukocyte antigen (HLA) region, is the main cause of HLA downregulation, occurring in the majority of invasive cervical carcinomas. In **CHAPTER 4** we investigated timing, frequency and mechanism of HLA class I downregulation in cervical carcinogenesis. To identify the stage of tumour development at which HLA class I aberrations occur, we selected 12 patients with cervical carcinoma and adjacent cervical intraepithelial neoplasia (CIN). Including the precursor lesions in our study permitted us to add to the current knowledge of HLA aberrations in invasive cervical carcinoma. We investigated HLA class I and β_2-microglobulin expression by immunohistochemistry in tumour and adjacent CIN. Loss of heterozygosity (LOH) was studied using microsatellite markers covering the HLA region. Fluorescence in situ hybridisation (FISH) with HLA class I probes was performed to investigate the mechanism of HLA loss. Immunohistochemistry showed absent or weak HLA class I expression in 11/12 cases. In 10 of these 11 cases downregulation occurred in both tumour and CIN. In 9/12 cases LOH was present for at least one marker in both tumour and CIN, 1 case showed only LOH in the CIN lesion and 1 case showed retention of heterozygosity (ROH) for all markers in both tumour and CIN. We concluded that HLA class I aberrations occur early and frequently in cervical carcinogenesis. This might allow premalignant CIN lesions to escape immune surveillance and progress to invasive cancer.

Loss of expression of the transporter associated with antigen processing (TAP) can influence HLA membrane expression which is frequently down-regulated in cervical cancer and its precursors. HLA class I molecules activate T-cells by antigen presentation and are therefore important for immunological surveillance. To add to the hitherto limited knowledge of molecular mechanisms underlying TAP loss in cervical cancer we investigated TAP expression, LOH and possible TAP mutations in **CHAPTER 5**. To identify the timing of changes in TAP expression 23 cervical carcinomas and adjacent precursor lesions were stained with HLA-A-, HLA-B/C-, β_2-microglobulin-, TAP1- and TAP2-specific MoAbs. TAP1 was not detectable in 10 out of 23 cervical carcinomas and 5 out of 10 adjacent CIN

lesions. All the lesions with low TAP expression also had altered HLA class I expression. To be able to separate tumour and non-tumour cells, cervical carcinoma samples were sorted by flow-cytometry and were subsequently analysed for LOH with markers in the TAP region on chromosome 6p21.3. LOH was found in 6 of the 10 lesions with TAP loss. Mutation analysis was then performed on these cases. In two cases we detected a polymorphism in the 5'-untranslated region (UTR) of the TAP1 gene. No mutations were detected. This study shows that there is altered TAP expression in a substantial number of cervical carcinomas. The underlying mechanism seems to be LOH in the TAP region, which is not accompanied by a mutation. In all cases with low TAP expression HLA class I loss was concomitantly detected, which supports previous reports on a strong association between TAP aberrations and loss of HLA class I expression.

Human papillomavirus (HPV) is a prerequisite for the development of cervical cancer. It has been established that multiple HPV infections are common in premalignant stages. Recently, multiple HPV infection in invasive cervical cancer has also been determined. In **CHAPTER 6** we investigated the significance of multiple HPV infections by studying their prevalence in cervical cancer samples from a low-risk (Dutch) and a high-risk (Surinamese) population and the correlation of HPV infection with tumour cell aneuploidy. SPF_{10} LiPA was used for HPV detection and typing in 96 Dutch and 95 Surinamese cervical carcinomas. Subsequently, samples with combined HPV 16/18 infections were sorted by flow cytometry and both diploid and aneuploid tumour cell fractions were HPV-typed by HPV 16- and HPV 18-specific PCR. HPV integration was investigated on the sorted cervical carcinoma cells. Fluorescent in situ hybridisation (FISH) on paraffin embedded tissue was modified to detect HPV 16 and 18 genotypes simultaneously and was performed on the sorted samples. Multiple HPV infections were present in 13.8% Dutch and 22.1% Surinamese HPV positive cervical carcinoma lesions. Three cases carried an HPV 16 and HPV 18 co-infection: in two cases, integrated HPV copies of either HPV 16 or 18 were detected in the aneuploid fraction, and in the third case both HPV 16 and 18 were present solely as episomes. These results show that multiple HPV infections are present in cervical cancer samples from both high- and low-risk populations. Multiple HPV types can be present in an episomal state in both diploid and aneuploid tumour cells, but integrated HPV genomes were detected only in the aneuploid tumour cell subpopulations.

Conclusions that were drawn and hypotheses that were developed are put into perspective in **CHAPTER 7**.