Biomolecular and epidemiological aspects of human papillomavirus induced cervical carcinogenesis

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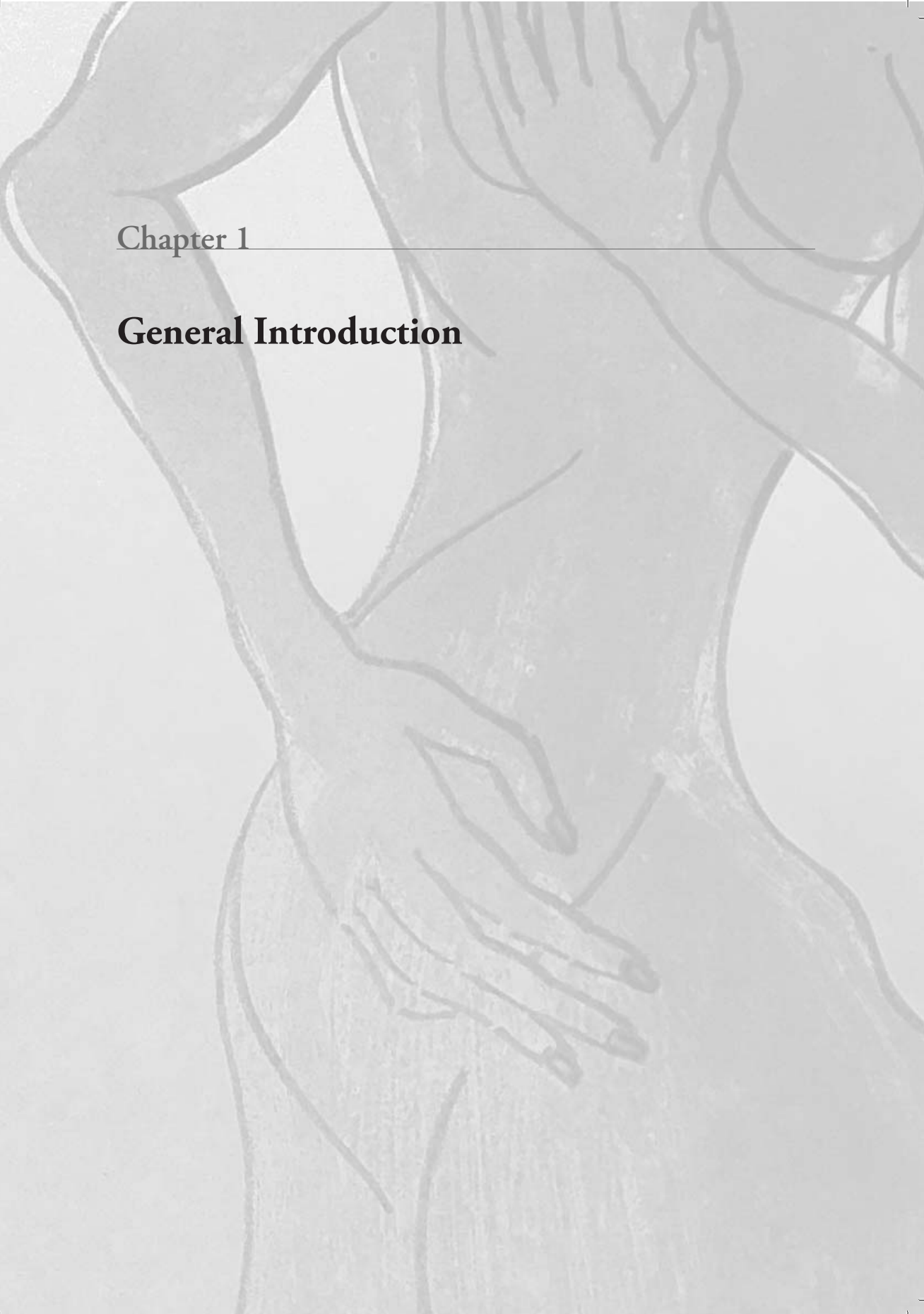

Chapter 1

General Introduction

Contents

1 Cervical Cancer

Aetiology

In 1842 Rigoni-Stern first mentioned that sexual intercourse and cervical cancer appeared to be related, because the disease was rare in nuns and common in prostitutes[1,2]. The idea that nuns, virgins and spinsters will not develop cervical cancer, despite being actively passed down through decades, was never scientifically well underpinned[3]. Nevertheless, epidemiological studies did show that cervical carcinoma was related to promiscuity and a young age of first sexual contact[4-6]. In 1976, Harald zur Hausen suggested that the development of cervical carcinoma was influenced by the sexually transmittable human papillomavirus (HPV), a virus until then only known to cause genital warts[7]. Several years later he first isolated, characterised and cloned HPV DNA from genital warts together with De Villiers and Gissman[8,9]. Since then, infection with human papillomavirus has been found to be the aetiological agent of cervical cancer[10-13]. The extensive HPV-mediated (cervical) carcinogenesis is elegantly investigated and summarised by Steenbergen[14].

Cervical carcinogenesis is a multistep process in which HPV infection is a necessary and early event. Other important steps are genetic changes and a failing immune system, which will be discussed in more detail in the following paragraphs.

Clinicopathology

The cervix uteri consists of the ectocervix and the endocervix, anatomically divided in the visible part (ectocervix) and the non-visible part (endocervix) of the cervix. The ectocervix is mainly lined with non-keratinizing stratified squamous epithelium and the endocervix with mucus producing columnar epithelium. The squamocolumnar junction (SCJ) is defined as the border between the two epithelia. In premenstrual women the SCJ is often located in the cervical canal, in the fertile years the SCJ is mostly located on the ectocervix. A physiological process called squamous metaplasia occurs in the cervix and arises from the subcolumnar "reserve cells". During this process columnar epithelium is gradually replaced by squamous cell epithelium. The SCJ shifts cephalad and in post-menopausal women it is located in the endocervix again. The area where the squamous metaplasia has taken place, which is the area between the original and the new SCJ, is called the transformation zone (**FIGURE 1***)*. The cells in this transformation zone are less stable and therefore particularly susceptible to viral infections. It is in this area where cervical carcinogenesis usually occurs[15-17].

A disturbed proliferation of squamous cells is called dysplasia or cervical intraepithelial neoplasia (CIN) and is the precursor of invasive carcinoma. The grading of CIN is based on the severity of the changes and especially on the proportion of the epithelial layer with neoplastic changes. In CIN I a third, in CIN II two third and in CIN III (almost) the total layer of epithelium contains atypical cells. Although CIN is a precursor lesion, the majority

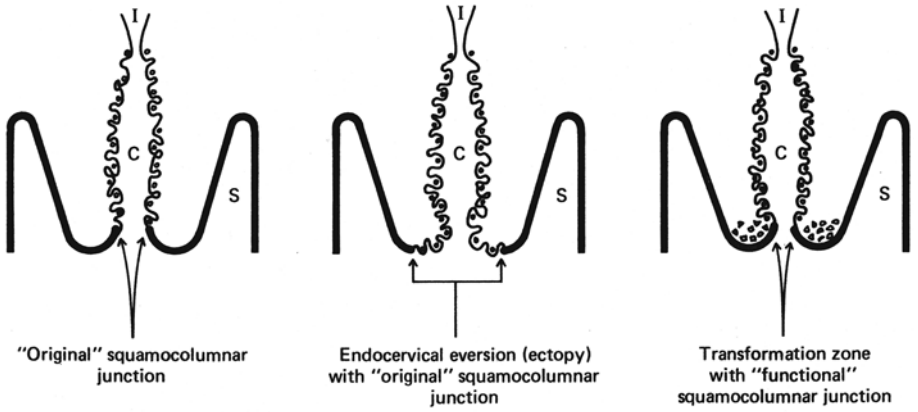

FIGURE 1
Squamocolumnar Junction and Transformation Zone
Adapted from[155]

of the untreated mild dysplasias persist or regress to normal cytology. The likelihood of progression of CIN I, CIN II and CIN III to invasive carcinoma ranges from 0.4 to 1%, 1.2 to 5%, and 3.9 to greater than 12%, respectively[18-20].

Different clinical (sub)stages of invasive cervical cancer are defined by the Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) as summarised in **TABLE 1**[21,22].

Several biological types of primary cervical neoplasms exist. Squamous cell carcinoma accounts for almost 80%, adenocarcinomas and adenosquamous carcinomas for most of

TABLE 1
FIGO stages, the different clinical (sub)stages of invasive cervical cancer as defined by the Fédération Internationale de Gynécologie et d'Obstétrique[21,22]

STAGE	SUBSTAGE
FIGO I – Limited to the uterus	**IA** - diagnosed only by microscopy • IA1 – stromal invasion < 3mm + ≤ 7mm spread • IA2 – stromal invasion 3-5mm + ≤ 7 mm spread **IB** - lesion with invasion > 5 mm or > 7mm spread • IB1 – lesion ≤ 4cm in greatest dimension • IB2 – lesion > 4 cm in greatest dimension
FIGO II – Invades beyond uterus	**IIA** – without parametrial invasion **IIB** – with parametrial invasion
FIGO III – extends to pelvic wall/ lower 1/3 vagina	**IIIA** – involves lower 1/3 of vagina **IIIB** – extends to pelvic wall and/or causes hydronephrosis or non-functioning kidney
FIGO IV	**IVA** – invades mucosa of bladder or rectum and/or extends beyond true pelvis **IVB** – distant metastases

the remaining 20%. Very rare types of epithelial tumours of the cervix are, for instance, glassy cell carcinoma and small cell carcinoma[23].

Treatment and Prognosis

The diagnoses CIN III or less depend on pathological findings. CIN III is treated by destruction or removal of the whole transformation zone. When the tumour is invasive the treatment of cervical carcinoma depends on its clinical (FIGO) stage. A uterus extirpation is usually the therapy of choice in case of micro invasive carcinoma (stage IA). When there is a wish for fertility in a woman with cervical cancer stage IA1 conisation is an option. In FIGO stage IB and IIA a radical uterus extirpation with (pelvic) lymphadenectomy or radiotherapy is performed. A more accurate staging of the tumour and estimation on prognosis is possible with surgical treatment. In addition, surgery permits the ovaries to be spared, which prevents fertile women from entering the menopause prematurely. A third advantage is the decrease in problems with sexual intercourse, possibly even less frequently arising if the radical surgery is nerve-sparing[24,25]. Postoperative radiotherapy is indicated with positive lymph nodes or positive surgical margins and parametrial involvement. In most clinics postoperative radiotherapy is also performed when other unfavourable prognostic factors are present, consisting of depth of tumour infiltration, lymphovascular space involvement or tumour volume. After randomised clinical trials the NCI now advises to treat the advanced stages (IIB-IV) and high-risk early stages with concomitant chemotherapy and radiotherapy[26].

Early stage cervical carcinoma can be treated successfully in the majority of the cases, with a 5-year recurrence-free survival (RFS) rate of 70-100%[26-28]. Survival for the more advanced stages varies and is influenced by lymph node involvement. The 5-year RFS is 50-70% for stages IB2, IIA and IIB, 30-50% for stage III and falls rapidly to 5-15% for stage IV[26]. Therapy for recurrent cervical cancer is generally disappointing and depends on previously performed radiotherapy. Less than 5% of these patients survive 5 years[26].

The most significant prognostic factor on survival is the FIGO stage, but other significant prognostic indicators exist as mentioned above[27-31]. In addition, a major prognostic factor is the level of development and poverty of the area in which the patient resides. The vast majority of the patients with cervical cancer cannot benefit from the advances of the last decades in treatment of this disease, because they live in impoverished countries with limited resources and no or inadequate screening programmes[26].

Epidemiology

Cervical cancer is the second most common cancer among females worldwide. Over 493,000 new cases are diagnosed yearly and it remains one of the leading causes of death from cancer among women[32,33]. The highest incidence rates are found in developing countries with age adjusted incidence rates up to 68.6 per 100,000 women[32]. In developed

countries the incidence rates have dropped to age standardised rates between 4.3 and 13.5[32,34-36]. In the Netherlands, an example of a low-risk country for cervical cancer, the age adjusted incidence and mortality rates per 100,000 women are 7.3 and 2.3, respectively[32].

The past decades both the cervical carcinoma incidence as well as the occurrence of the advanced FIGO stages have decreased 30-60% in developed countries. Screening programmes in developed countries might account for the majority of this decline in cervical carcinoma incidence and mortality rates although the impact has never been studied in randomised trials[35-37].

Prevention

Prevention of cervical cancer can be accomplished by implementing well organised, population-based screening programmes. The present screening programmes aim to trace cervical precursor lesions by cytologically analysing cervical smears. Several classification systems exist for recording cytological abnormalities, including the Bethesda System[38] and the Papanicolaou Classification[39] (**TABLE 2**). In the Netherlands cervical cytological abnormalities are graded using the KOPAC system, the official Dutch microscopical coding system[40,41]. This system allows for simultaneously scoring of inflammatory and (pre) neoplastic changes. A Pap score is given for communication with clinician and patient.

Nowadays, the developed countries all have effective screening programmes with a coverage and attendance of 50-80%[36]. In most of these countries a cervical smear is taken every three or five years and targets women aged between 30 and 55. Developing countries remain high-risk areas for cervical cancer. They account for 79% of the cervical cancer incidence worldwide and advanced FIGO stages are still of frequent occurrence in these countries[35,36]. Therefore, implementation of screening programmes in developing countries seems an appropriate measure to decrease the high incidence.

TABLE 2

Description, various classification systems and translation of codes for normal squamous epithelial cells and (pre)neoplastic changes

DESCRIPTION	PAPANICOLAOU	BETHESDA	KOPAC P-Code
Normal	Pap I	Normal	P1
Borderline Changes	Pap II	ASCUS	P2-3
Mild Dysplasia	Pap IIIA	(L)SIL	P4
Moderate Dysplasia	Pap IIIA	(H)SIL	P5
Severe Dysplasia	Pap IIIB	(H)SIL	P6
Carcinoma in Situ	Pap IV	(H)SIL	P7
Micro invasive Carcinoma	Pap V	Carcinoma	P8
Squamous Cell Carcinoma	Pap V	Carcinoma	P9

Cervical Cancer in Suriname

Suriname is a high-risk area for cervical carcinoma with an incidence of 27 per 100,000 women[32]. A three- to sixfold higher percentage of the advanced FIGO stages (IIB-IV) is established compared to the Netherlands, a low-risk country for cervical cancer. There are various ethnicities living in Suriname, which have different cervical carcinoma incidence rates[42]. These ethnicities are the Creoles, the Hindustani, the Javanese, the Maroons, the Amerindians, the Chinese and all possible mixtures of these ethnicities. Hitherto, the high cervical cancer incidence in Suriname and other high-risk countries is attributed to absence of an organised screening programme, a presumed high(er) prevalence of the human papillomavirus (HPV), immunological factors and environmental or cultural based factors, but more research is still needed.

2 Human Papillomavirus (HPV)

Biological Aspects

(Human) Papillomavirus is a genus of the family Papovaviridae. The HPV virions are non-enveloped and icosahedral with a circular double stranded DNA (dsDNA) genome of almost eight kilo bases in length. The dsDNA consists of six open reading frames (ORF) encoding early (E) proteins, two ORFs encoding late (L) proteins, and a non-coding long

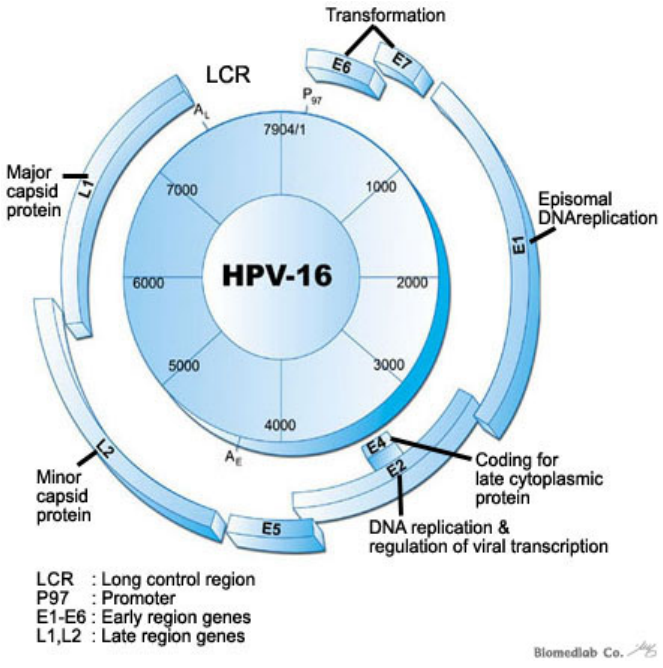

FIGURE 2
The HPV Virion

control region (LCR) (**FIGURE 2**). Papillomaviruses are classified based on their degree of DNA homology in the nucleotide sequences of E6, E7 and L1 ORFs.

Cervical HPV infection occurs through microabrasion of the genital epithelium allowing access of the viral particles to target cells. For a lesion to persist, it is suggested that the virus has to infect an epithelial stem cell[43-45]. It is generally thought that expression of the viral E1 and E2 proteins maintains the HPV DNA as an episome and facilitates the correct segregation of genomes during cell division[45-47]. The major viral oncoproteins E6 and E7 have been shown to play a vital role in viral episome persistence by interfering with the cell cycle[48,49]. They can stimulate cell cycle progression and associate with cell cycle regulators[50-52]. E6 binds to p53 and herewith inactivates p53-mediated growth suppression and apoptosis[53], whereas E7 binds to pRb which inactivates this negative regulator of the cell cycle[54].

To date, 118 papillomaviruses (PVs) comprising of 96 human and 22 animal papillomavirus types have been completely described and several hundred putative new PVs types are partially characterised[55-58]. The HPV genotypes can be divided into a subgroup

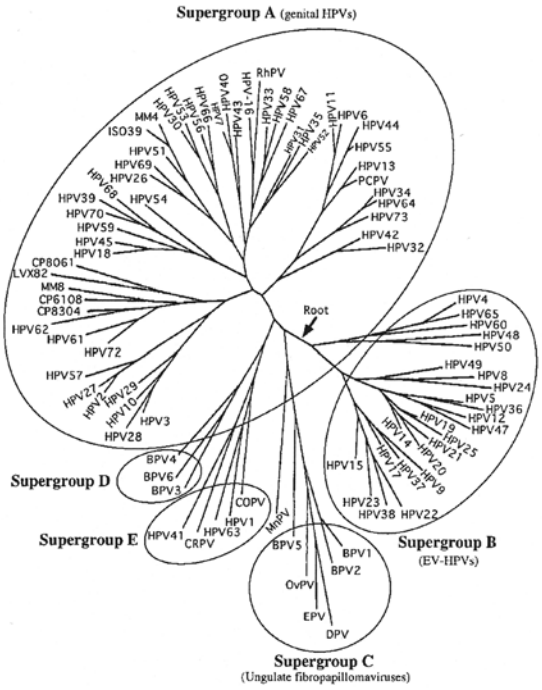

FIGURE 3
Phylogenetic Tree of Papillomaviruses
Adapted from[156]

of mucosal HPV types, which is associated with anogenital lesions and a subgroup of cutaneous HPV types, which induce mostly benign skin lesions. The mucosal HPV types are further classified as (probable) oncogenic or high-risk types which are predominantly found in CIN III lesions and anogenital cancers, and low-risk HPV types which are mainly found in benign and CIN I-II lesions[57-59](**FIGURE 3**). Forty types infecting the anogenital tract are found in anogenital cancer specimens[56,60,61].

Occurrence

Most women undergo an HPV infection during life, but are able to clear it without ever having any clinical symptoms. HPV DNA is detectable in 2% to more than 20% of the global female population at any time[33]. In women with normal cytology or mild dysplasia the predominant HPV types are low-risk. With increasing severity of dysplasia the overall HPV prevalence also increases and the oncogenic HPV types become more prevalent. Finally, in invasive cervical carcinoma the oncogenic HPV prevalence is established to be almost 100% and thus HPV is accepted to be a necessary cause[10-13,33,62-64].

The prevalence and distribution of HPV genotypes show considerable geographic and ethnical variation, especially for the less common types. In most areas the predominant HPV genotypes in cervical cancer are HPV 16 (30-50%) and HPV 18 (10-15%). In non-western countries other types, like 45, 52 and 58, are also detected in a considerable proportion of the cervical cancers[65,66].

It is possible to have an HPV infection with multiple HPV genotypes simultaneously. Different studies report about multiple HPV infections in cervical samples with normal cytology or atypical squamous cells of undetermined significance (ASCUS) and mild to severe dysplasia[67-72]. It is generally thought that the cells infected with the most oncogenic type will eventually transform into the invasive tumour clone. In the majority of invasive carcinomas mainly single HPV infections were detected and until recently only occasionally a multiple HPV infection was found. Because of newly developed techniques better suitable for detection of multiple HPV types, it is now possible to get an accurate indication of their prevalence in cervical carcinoma and its precursors.

Detection Techniques

The (human) papillomaviruses can only replicate in differentiating stratified squamous epithelium, which cannot be grown as a conventional cell culture. Serological tests for HPV have an estimated sensitivity of only 50% using detection of HPV DNA as a standard[73]. Therefore HPV infection and typing can only be accurately diagnosed by molecular methods[73,74]. Several HPV assays are described, but nowadays the polymerase chain reaction (PCR) based techniques are the method of choice due to the greater sensitivity and technical facilities[75]. Since there is significant sequence variation between the genotypes, either a large number of type-specific PCRs or a single broad-spectrum PCR primer set,

can be used. Several general PCR primer sets have been developed, which aim at the most conserved sequences of the viral genome, permitting amplification of a broad spectrum of HPV genotypes[70,73,76-78]. After the HPV detection, the HPV genotyping is performed by sequence analysis, a reverse hybridisation assay[71,79-81] or, more recently, micro arrays[82,83].

3 Immunology and Cervical Carcinoma

Human Leukocyte Antigen (HLA)

Background

The immune system is the specific defence mechanism against the external world. It comprises the antibody mediated (humoral) system, for which B-cells are responsible, and the cellular system, predominantly mediated by cytotoxic T-lymphocytes (CTLs). Both systems are involved in the immunological management of a viral infection. The humoral immune system probably is important for prevention of viral infections, the cellular immune system for the elimination of a virus and virus induced lesions. Immunological surveillance in HPV associated lesions is thus performed by CTLs, which are activated when foreign (antigenic) proteins are presented to the CTL receptor by human leukocyte antigen (HLA) class I. HLA class I molecules are expressed on virtually all cells[84].

HLA

The major histocompatibility complex (MHC) is located on the short arm of chromosome 6 at 6p21.3 and comprises 240 different gene loci[85], of which many encode for HLA molecules. The MHC can be subdivided into three closely linked multigene families, class II (HLA-DR, -DP and -DQ genes), class III (includes genes encoding complement and tumour necrosis factor (TNF)) and class I genes (the classical class IA genes, HLA-A, -B and -C, and the non-classical class IB genes, HLA-E, -H, -G and -F) (**FIGURE 4**). The MHC genes all encode for proteins that control the immune responses to pathogens, graft acceptance or rejection and tumour surveillance. The HLA class I and class II molecules are encoded by, respectively, class I and class II genes. On each chromosome 6 the genes in the class I-III regions compose a combination, called a haplotype. The two haplotypes on the chromosome 6 pair combined are called the HLA genotype. The HLA genotype is expressed as HLA class I and class II molecules on the cell surface and this is called the HLA phenotype.

HLA Class I Antigen Processing and Presentation

HLA class I molecules are expressed on nearly every somatic cell[84] and on virally infected tumour cells. They consist of a polymorphic heavy α chain (HC), encoded by the HLA class I genes HLA-A, -B and -C on chromosome 6p21.3, in non-covalent association with

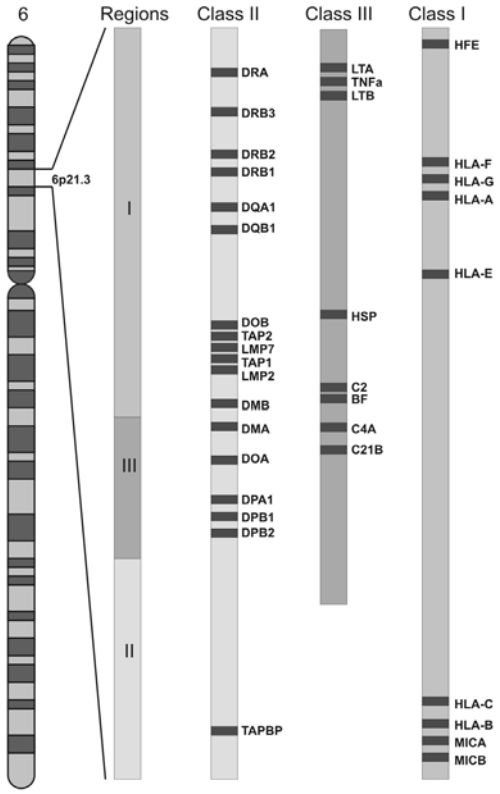

FIGURE 4
The Major Histocompatibility Complex. Provided by E.S. Jordanova

the light β chain, encoded by the β_2-microglobulin (β_2m) gene on chromosome 15q21. The association with β_2m is important for the stability of the HLA class I molecule[86].

The antigen processing and presenting by the HLA class I molecule or HC-β_2m complex concerns mainly endogenous processed antigens (viral or tumour associated products, or waste products from the cell itself). Endogenous proteins are degraded in the cytosol into smaller fragments, called peptides. These peptides are subsequently transported by the transporter associated with antigen processing (TAP), which consists of two subunits TAP1 and TAP2 that form a channel in the endoplasmatic reticulum (ER)-membrane[87]. In the ER, the assembly of the HLA class I heavy chain, the β_2m light chain[88] and the peptides[89,90] is chaperoned by several proteins[91-95]. The newly formed complex is then transported via the Golgi network to the cell surface and is subsequently presented to circulating CTLs (**FIGURE 5**). In addition, TAP independent mechanisms have been described[96-101].

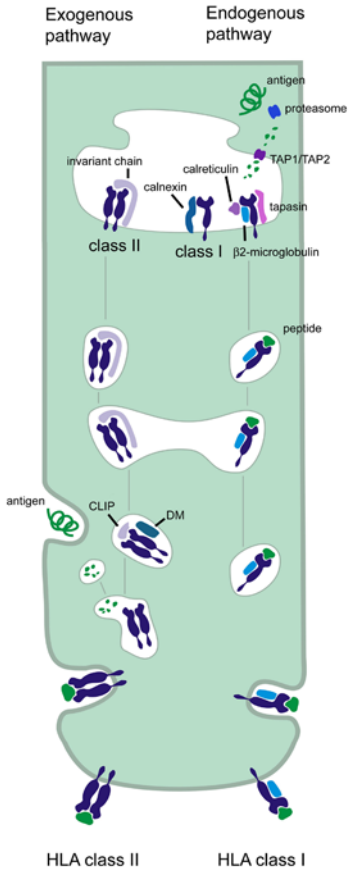

FIGURE 5
HLA Class I Antigen Processing and Presentation. Provided by E.S. Jordanova

Immune Evasion in Cervical Cancer

Loss of HLA class I cell surface expression, HLA class I downregulation, occurs in various solid tumours and tumour cell lines[102,103] and is thought to result in escape from the cytotoxic CTL attack. It occurs frequently in cervical carcinoma and is predominantly caused by losses at chromosome 6p21.3, the region where the HLA genes are localised[104-107]. HLA class I expression was also tested in CIN-lesions with varying outcomes[108-110], but in these lesions knowledge remains limited about altered HLA class I expression in association with the underlying mechanisms. HLA class I downregulation is frequently associated with impaired TAP expression[111-114] and it has been correlated with TAP gene regulatory abnormalities and mutations in various tumour types[115-120].

Currently, research concerning prevention and treatment in cervical cancer focuses on HPV vaccines[121-124]. Therapeutic vaccines are based on the viral oncogenic proteins E6 and E7 and aim to induce cell-mediated responses to eliminate the transformed tumour cells[124]. CTLs will only recognise viral peptides if HLA class I molecules present them on the surface of the infected cell. Therefore, HLA class I downregulation would compromise the effectiveness of an HPV vaccine.

Genetic Basis of Immune Evasion

During carcinogenesis multiple genetic events take place involving proto-oncogenes and tumour suppressor genes (TSGs), two classes of genes that are both involved in tumour progression and metastasis[125-128]. Vogelstein and Kinzler described the multistep nature of cancer[125], which is distinctly illustrated by the multigenic model for colorectal tumorigenesis[129]. It was suggested that only a subset of genetic pathways can initiate the tumorigenic process in particular cell types and that mutation at some genes confers a selective growth advantage[125]. In cervical cancer the two HPV-encoded oncoproteins E6 and E7 can independently induce chromosomal abnormalities, which causes genomic instability and ultimately facilitates carcinogenic progression[130,131].

Alfred Knudson advanced his "two hit" model in 1971 as a necessary condition for certain cancers to develop[132]. All chromosomes exist in pairs and carry the genes, of which most have two similar copies. An alteration in each of two gene alleles inactivates a tumour suppressor gene, leading to tumour development and growth. One hit is an innate (germ line) mutation (occurs in hereditary cancer) or a somatic mutation (in sporadic cancer), the other hit an event that often leads to loss of heterozygosity (LOH)[133-137]. Such an event can be deletion, gene conversion, (mitotic) recombination, translocation, nondisjunction or chromosome loss, chromosome duplication and promoter methylation and could lead to haploinsufficiency[128,137-139]. LOH can be detected by polymorphic repeat markers flanking the locus of interest, or situated in the target gene. Those polymorphic markers are formed based on repeat sequences in the DNA, which are heterozygous for the two gene alleles in a large percentage of the population. The LOH analysis is used to indicate loci that may contain a TSG. However, accurately defining a common LOH region with a possible TSG can be confounded by deficient LOH detection, genetic instability and inter-/intratumour heterogeneity[139].

LOH at chromosome 6p21.3, the region where the HLA genes are located, occurs at high frequencies in cervical cancer[140-146]. With most genes both alleles need to be switched off to inactivate the gene. HLA genes are co-dominant therefore switching off one gene allele could induce inactivation. Koopman *et al.* proved in a study on fresh tumour tissue that LOH at 6p21.3 represents an important and common mechanism by which HLA genes and their products are abolished[107]. LOH on 6p21.3 is also frequently detected in high grade CIN-lesions, indicating that it is an early event in the cervical carcinogenesis[147,148]. A

genetic basis was shown for most of the cervical tumours with an altered HLA phenotype. This involved, besides LOH, class I gene mutation (on chromosome 6p21.3) and β_2m mutation (on chromosome 15q21) or a combination of these events[107,149]. Further investigations are yet needed of the unexplained HLA class I phenotype alterations to clarify the underlying mechanisms.

Other mechanisms causing HLA class I downregulation could be mutation(s) and LOH in the genes encoding for TAP I or II. A recent cervical carcinoma study reported possible mutations in these TAP encoding genes, but the method of detection was not conclusive. In fact, LOH and polymorphisms in TAP genes were studied and loss of TAP expression was not investigated[150].

Vaccination

The close relationship between viral infection and cancer makes HPV an attractive target for prophylactic and therapeutic vaccine development. Prophylactic vaccines are developed to prevent infection by generation of antibodies to recombinant capsid proteins L1 (and L2) that neutralise viral infection[121,122,124,151]. Therapeutic vaccines generally target E6 and E7 which are critical for the immortalisation in (pre)malignant cells in order to induce regression of established infection and possibly control the HPV-associated lesion[121,123,124,152]. The vaccines can be delivered directly as protein, as DNA that encodes and expresses the requisite viral protein(s), or by heterologous viral vectors[153]. Various approaches are being taken in the development of prophylactic HPV vaccines, the most advanced and promising being the use of non-infectious recombinant virus-like particles assembling from pentamers of the L1 capsid protein and inducing high titres of virus-neutralising antibodies[124]. Encouraging results from animal and human vaccine trials have led to large scale efficacy trials concerning prophylactic and therapeutic vaccination[121-124,151]. Recent research on safety and efficacy of candidate prophylactic vaccines have shown a nearly 100% protection against the development of (high-grade) HPV 16 and 18 induced cervical lesions[124,151,154]. Several therapeutic vaccines have been developed and are currently under clinical evaluation[124].

4 Scope of this Thesis

As discussed previously, cervical cancer is preceded by several stages of precursor lesions. Population-based screening programmes aim to trace these precursor lesions by cytologically analysing cervical smears. The premalignancies are mainly induced by HPV infection, which is very common in young women worldwide and influenced by endogenous and environmental factors. Behavioural factors like lifestyle and viral characteristics are important environmental factors. Most HPV infections are transient and are cleared within months as a result of an effective host immune response. Clearance of oncogenic

HPV infection is accompanied by cytological regression, which occurs in the majority of mild cervical abnormalities. The cellular immunity is an important effector mechanism for the clearance of established HPV infection and thus it is likely that the immunological surveillance by CTL responses plays a role in the protection against the development and progression of cervical lesions. CTL responses are generated when foreign (antigenic) proteins are presented to the CTL receptor by HLA class I molecules. TAP is physically associated with HLA class I molecules and is required for the transport and processing of the viral or tumour antigens degraded to peptides.

HLA or TAP aberrations might lead to a failing immunological surveillance, which allows for the oncogenic HPV infection to become persistent. Persistent infection with oncogenic HPV types is essential for the development and progression of cervical dysplasia and, finally, for the development to cervical cancer. It is accepted that HPV is present in all cervical carcinomas, which could be in episomal and integrated form. Viral integration of the HPV in the human genome appears to increase with progression to cervical cancer, but the biological significance is still debated. Occasionally cervical carcinoma is infected with multiple HPV types. Limited knowledge exists of multiple HPV infections in cervical cancer and it is complicated to investigate due to technical difficulties.

The past decades both the cervical carcinoma incidence as well as the occurrence of the advanced FIGO stages have decreased in developed countries. This is predominantly due to the implementation of well-organised screening programmes. The population based screening selects women at risk of developing cervical cancer and prevents it by treating women with moderate and severe dysplasia. In addition, it allows for downstaging of the disease by capturing cervical carcinoma patients in the presymptomatic stages. Unfortunately, cervical carcinoma remains the major cause of cancer related mortality among women in developing countries. Implementation of screening programmes in developing countries therefore seems an appropriate measure to decrease the high incidence.

In **CHAPTER 2** we analysed cervical smears of four different Surinamese ethnicities to determine the prevalence of cytological abnormalities of women attending the first organised screening programme in a high-risk area for cervical cancer. In addition, we investigated whether the differences in cervical cancer incidence existing between the studied ethnicities was reflected in the proportions of cytological abnormalities.

It is valuable to obtain insight in the relative influence of endogenous and environmental factors on differences in cervical carcinoma incidence rates between high- and low-risk areas. This could be achieved by comparing the cytological abnormality incidence rates of immigrants from a high-risk area for cervical cancer with those of the source population. In **CHAPTER 3** we therefore compared cervical cytological abnormality incidence rates in Surinamese women living in Suriname and the incidence rates in the Surinamese immigrants living in the Netherlands. This scenario factors out endogenous differences, as the same ethnic population has been studied in two areas.

As previously discussed, immune surveillance for HPV associated lesions is performed by CTLs, which are activated by the antigen presentation of the human leukocyte antigen (HLA) class I molecules. In cervical cancer HLA class I aberrations are common. To determine the timing, frequency and mechanism of HLA class I downregulation in cervical carcinogenesis, we performed immunohistochemistry, loss of heterozygosity (LOH) analysis and fluorescent in situ hybridisation (FISH) on cervical carcinoma specimens and adjacent cervical intraepithelial neoplasia (CIN) lesions **(CHAPTER 4)**.

The frequently occurring HLA aberrations in cervical cancer are predominantly caused by extensive LOH at chromosome 6p21.3, partially in combination with mutations in β_2m or HLA class I genes. The significance of disturbed transporter function in cases with loss of HLA class I expression that could not be explained, needs to be explored. Low transporter associated with antigen processing (TAP) expression has previously been reported and associated with HLA class I downregulation in cervical carcinomas, but limited information exists about underlying mechanisms. In **CHAPTER 5** we investigated loss of TAP and HLA class I expression in invasive cervical carcinoma and adjacent precursor lesions, to determine the occurrence of TAP downregulation and its relation with HLA class I in cervical carcinogenesis. In addition, we examined possible causative mechanisms of the TAP downregulation by performing LOH and gene mutation analysis.

Up until now, it was the common opinion that, although precursor lesions may have multiple human papillomavirus (HPV) infections, invasive cervical carcinoma is a clonal process and therefore infected with only one HPV genotype. Recently, a technique better suited for detection of multiple HPV infections was developed. This permitted us to investigate the prevalence of multiple HPV infections in cervical cancer for a low-risk (Dutch) and a high-risk (Surinamese) population. Additionally, we examined whether cervical carcinomas with a multiple HPV infection are derived from one malignant clone infected with multiple HPV types or alternatively, whether multiple malignant clones developed to invasive carcinoma **(CHAPTER 6)**.

In **CHAPTER 7** several of the topics that are dealt with in this thesis are highlighted in a general discussion. Finally, the findings described in the aforementioned studies are summarised in English and Dutch **(CHAPTER 8 and 9)**.

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