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Transforming Growth Factor beta-1 in cervical cancer

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General Introduction

INTRODUCTION

Epidemiology

Carcinomas of the genital tract – particularly cancer of the cervix – account for almost 12% of all cancers in women, after breast cancer representing the second most frequent malignancy in the world.[1] Worldwide cervical cancer will develop in about 400,000-500,000 women yearly (incidence 14,2/100,000). and about 190,000 women will die of the disease per year.[2-4] Nearly 80% of all cases arise in less developed countries, with the highest incidence rate in Zimbabwe (67,21/100,000) and the lowest in China (2,64/100,000). In the Netherlands, the incidence rate is 9/100,000 (around 700 new patients a year) and the mortality rate 3/100,000 (around 250 women a year).[5] During the last 50 years, cervical cancer incidence and mortality has decreased by more than 75%, primarily because there has been a natural decline in the western world over this period. Nowadays, in the developed world, cervical cancer is the fourth frequent prevalent cancer in women, after breast, colorectal, and endometrial cancer. However, the major drops have only occurred in those countries with a comprehensive screening program, which contrasts with the developing world –including South Asia, sub-Saharan Africa and South America[6]. The median age at diagnosis is between 45 and 65 years. In the Netherlands we observe a peak incidence at 35 years and at 55 years. Women above 55 years contribute disproportionably to cervical cancer mortality, primarily as a result of more advanced disease at diagnosis.[7]

Etiology and risk factors

The cervix uteri is covered with columnar epithelium, which lines the endocervical canal, and squamous epithelium, which covers the ectocervix. The point where they meet, so called the squamous columnar junction (SCJ), is a dynamic point that changes in response to puberty, pregnancy, menopause and hormonal stimulation. In neonates, the SCJ is located on the ectocervix and at menarche subcolumnar reserve cells are stimulated, by a change of vaginal pH, to undergo metaplasia. The metaplasia advances from the original SCJ inwards and the area in which this process establishes is called the transformation zone. It is at that site where cervical premalignant and malignant lesions predominantly originate.[6] Several risk factors for cervical carcinoma have been identified, including (persistent) HPV (Human Papillomavirus) infection, early sexarche (< 16 years), multiple sexual partners (> 4), smoking, and perhaps even exposure to environmental tobacco smoke, the duration of use of hormonal contraceptives, a low socio-economic status, high parity and an immunocompromised status.[7-13] Many

of these risk factors are linked to sexual activity and exposure to sexually transmitted diseases and most of them are the same for squamous cell carcinoma and adenocarcinoma, with the possible exception of smoking.[12] Cervical cancer does not appear to segregate in families in such a way that would lend support to the existence of a host genetic factor of major importance such as in familial breast and ovarian cancer. Nevertheless, some large Swedish epidemiological studies show a significantly increased relative risk for biological full-sisters of patients of 1.93 and for biological mothers of 1.83 compared to a relative risk of 1.45 for biological half sisters and 1.10 and 1.15 respectively for adoptive mothers and sisters (both not significantly different from no risk increase). In those families the age of onset also is slightly lower than in sporadic cases.[14,15] Possibly this has to do with environmental factors (such as smoking) and/or genetic susceptibility to HPV infections.

Infection with one of the oncogenic HPV types has been established as the critical step in the development of cervical cancer; HPV DNA is detectable in virtually all cervical cancers.[11-13,16] The multi step process of the cervical carcinogenesis model, which is discussed in detail in a next paragraph, involves HPV infection and via development of cervical intraepithelial neoplasia (CIN) 1,2 and 3 finally (micro) invasive carcinoma and metastasis. The concept of CIN was introduced in 1968 by Richart, who indicated that all dysplasias have the potential for progression.[17] The degree of neoplasia (CIN 1 to 3) is based on the extent of mitotic activity, immature cell proliferation and nuclear atypia: when these are present only in the lower one third of the epithelium, the lesion is designated CIN 1. Involvement of the middle and upper thirds is diagnosed as CIN 2 and CIN 3. It is assumed that about 60% of CIN 1 will regress spontaneously, 30% will persist and 10% will progress to CIN 3 and 1% to invasion. Of CIN 2 approximately 40% will regress, 40% will persist, 20% will progress to CIN 3 and 5% to invasive carcinoma. CIN 3 will regress in roughly 30%, persist in approximately 60% and proceed to invasion in 12% of cases.[6-18] Chan et al. demonstrated that absence of persistent HPV-infection, either initially or after spontaneous resolution, and number of sexual partners ≤ 5 were significant predictors for spontaneous regression of CIN 2 and 3 after initial biopsy.[9] The multistep process from HPV infection to invasive carcinoma can take 15 years or more, since the average age of patients with CIN is 20-30 years and the average age at which invasive cancer is detected 45-65 years. However, nowadays it is also being proposed that the development of invasive carcinoma does not always follow the model proposed by Richart, but that infection with an oncogenic HPV can result in a quicker and more direct process of invasive growth.

Histopathology

Using the WHO classification, about 80% of epithelial cervical tumors are squamous cell carcinomas, 10-15% are adenocarcinomas and the rest group of the epithelial tumors consists of the adenosquamous carcinoma, the adeno cystic and the adenoid basal carcinoma and the neuroendocrine tumors.[19]

The squamous cell carcinoma is classified as keratinising -containing keratin pearls composed of circular whorls of squamous cells with central nests of keratin-, non-keratinising –in which individual cell keratinisation may occur, but keratin pearls are absent-, and rare variants such as basaloid, verrucous, warty, papillary or lymphoepithelioma-like carcinomas. Histologically the tumor cells form irregular infiltrative nests surrounded by stroma, which is usually infiltrated by a variety of immune cells. Special mucin staining like Periodic acid-Schiff (PAS) staining is required to confirm the adenosquamous subtypes.[16,20] The carcinomas in our study consist of squamous cell carcinomas (87%), adenocarcinomas (7%) and adenosquamous carcinomas (6%).

Clinical staging, treatment modalities and prognosis

Cytological screening to detect premalignant lesions or cancer of the uterine cervix has been shown to decrease both the incidence and mortality for this disease in those areas where it has been extensively applied.[14-16] To characterize cytomorphological abnormalities in exfoliated cells of the cervix, the PAP-classification is being used, which corresponds to histological changes of the cervix. In the Netherlands the smears are assessed by a quality norm, the KOPAC-B system, in which the composition of the smear, the presence and type of infection, the squamous cells, the endometrial cells, the columnar cells of the endocervix and the adequacy of the specimen are judged.[14] Analogous to this in the USA the Bethesda system has been developed.[21,22] Both scoring systems with corresponding histological diagnosis are represented in Table 1. In case of repeated PAP 2 or 3A and one PAP 3B or higher colposcopy with biopsy will be performed. If histology shows CIN 1, treatment will be conservative with follow-up smears. CIN 2 and 3 are usually treated by large loop excision of the transformation zone, followed by cytological screening.

Cervical cancer staging is, in contrast to other cancers that are staged surgically, a clinical staging. The FIGO (Federation Internationale Gynecologique et Obstetrique) system of classification of cervical cancer (Table 2) is originally based on the results of clinical examination, essentially of the anatomical extent of disease, and is determined at the time of primary diagnosis.[15] In case of doubt about the clinical stage the lowest FIGO stage is chosen. Although the clinical and the surgical (TNM) staging only correlate for approximately 60%, the clinical

Table 1 - Cytological and histopathological nomenclature.

Papanicolaou Cytology	Histological equivalent	Bethesda
Pap 0 Specimen unsatisfactory for evaluation	-	-
Pap 1 normal	-	Within normal limits
Pap 2 Benign atypia (atrophia or atypic tissue regeneration)	-	ASCUS
Pap 3A1 Mild dysplasia or atypia*	CIN 1	Low-grade SIL
Pap 3A2 Moderate dysplasia or atypia	CIN 2	High-grade SIL
Pap 3B Severe dysplasia or atypia	CIN 3	High-grade SIL
Pap 4 Carcinoma in situ	CIN 3	High-grade SIL
Pap 5 (microinvasive) cancer	(microinvasive) cancer	(microinvasive) cancer

Dysplasia refers to changes in squamous epithelial cells, atypia to changes in columnar epithelial cells. CIN: cervical intraepithelial neoplasia. SIL: squamous intraepithelial lesion. ASCUS: atypical squamous cells of undetermined significance.

Table 2 - Fédération Internationale Gynécologique et Obstétrique.

FIGO stage	TNM classification	
	T0	No primary tumor
0	Tis	Carcinoma in situ
T_1	T_1	Tumor confined to the cervix
1A1	T1a1	Infiltration depth < 3 mm, linear extension < 7 mm
1A2	T1a2	Infiltration depth < 5 mm, linear extension < 7 mm
1B1	T1b1	Tumor > 1A, but < 4 cm
1B2	T1b2	Tumor > 1A and > 4 cm
2	T2	Tumor extended beyond the cervix, but not yet on to the pelvic wall or the lower one-third of the vagina
2A	T2a	Tumor extended beyond the cervix in the upper two-thirds of the vagina, no parametrial involvement
2B	T2b	Obvious parametrial involvement, not yet on to the pelvic wall
3	T3	Tumor involves parametrium on to the pelvic wall and/or the lower one-third of the vagina and/or causes hydronephrosis or non-functioning kidney
3A	T3a	Tumor involvement of the lower one-third of the vagina
3B	T3b	Lateral extension on to the pelvic wall and/or nephrosis or non-functioning kidney
4	T4	Tumor spread beyond the pelvis
4A	T4a	Tumor clinically involves the mucosa of the bladder or the rectum
4B	T4b	Spread to distant organs

stage can not be changed anymore after treatment. Cervical cancer spreads per continuum in the surrounding tissues or via the lymphatic route: the parametrial, internal iliac (obturator/hypogastric), external iliac, presacral and common iliac lymph nodes. Para-aortic nodes are second station and are considered distant metastases. The most common sites of distant spread besides the aortic nodes include mediastinal nodes, the lungs and skeleton. The optimal treatment of cervical cancer varies with the stage at diagnosis. Briefly, stage 1A1 tumors can be treated by cone biopsy and careful observation or vaginal or abdominal hysterectomy. FIGO stage 1A2 tumors are best treated by radical hysterectomy with bilateral pelvic lymphadenectomy, as are stage IB and IIA tumors. Depending on the histopathological findings, which include tumor positive lymph nodes, tumor positive surgical margins and parametrial involvement, post surgical radiotherapy consisting of brachytherapy and external beam radiation, possibly combined with chemotherapy (chemo radiation), is given. Alternatively, stages IA2-IIA can primary be treated by radiotherapy. In women with small lesions (< 2cm) who wish to preserve their fertility radical trachelectomy with pelvic lymphadenectomy is a new alternative treatment modality. Patients with stage IIB, stage IIIA, IIIB and IVA are usually primarily treated by radiotherapy, mostly in combination with cisplatin-based chemotherapy or hyperthermia, since the combination of these has been shown to be superior to radiotherapy alone for bulky, locally advanced disease.[16,23-25] Treatment of stage IVB disease is individualized and can consist of palliative radiation of the pelvis or metastatic sites and/or chemotherapy.
The 5-year survival of stage 1A is about 95%; stage 1B/2A 80-90%; stage 2B 65%; stage 3 40% and stage 4A < 20%.[25] Prognostic unfavourable factors are lymph node metastases, tumor volume > 40 mm, infiltration depth > 15 mm and vaso invasion. 5 yr survival in stage 1B/2A drops from over 80 % to 55% in case of lymph node metastasis.[26] Approximately 30 % of women with invasive cervical cancer die from recurrent or persistent disease after initial therapy.[25] Treatment of recurrent disease depends on the mode of primary therapy and site of recurrence. (Chemo-) radiation, if not already administered in maximum dose earlier, can lead to response rates of 20%-60% in stage I-IIA patients primarily treated with radical hysterectomy.[27] When prior radiotherapy has been given, pelvic exenteration can be performed if the tumor has not extended to the pelvic sidewall and there are no distant metastases. 5 yr survival after anterior exenteration is 33-60% and 20-46% for those women undergoing total exenteration.[28,29] Chemotherapy is not considered curative in recurrent disease, also because of poor blood supply in a prior radiated field. Best results might be expected in patients with chest metastases.[30]

MULTISTEP CERVICAL CARCINOGENESIS

HPV; infection and integration

In 1976 the first report was published on the appearance of koilocytes in cervical smears and mild dysplastic cervical lesions to indicate the presence of a papilloma virus infection.[31] The first HPV types were isolated directly from cancer biopsies of the cervix – HPV 16 and 18 were cloned in 1983 and 1984, respectively – which initiated a rapid expansion of the field.[32,33] To date 150 genetically distinct types of HPV have been identified. DNA sequences of the 'high-risk' types 16 and 18 and, in descending order of frequency, types 45, 31, 33, 52, 58, 35, 59, 56, 39, 51, 73, 68 and 66 are found as a single or multiple infection in more than 99% of all cervical carcinomas and the majority of the high grade precursor lesions.[6,12,34] The 'low-risk' HPV-types found primarily in genital warts and non-malignant lesions consist of type 6 and 11 and, less prevalent, type 42, 44, 53 and 83.

All papillomavirus types possess a circular double-stranded DNA genome, containing 7000-8000 bp.[35] It harbors a number of genes coding for early functions (E1-E8), the non-structural proteins that are not equally represented among different HPV types, as well as two genes encoding the viral structural late proteins L1 and L2. The early genes E1 and E2 play an important role in viral DNA replication, E2 specifically also in the transcriptional regulation of the viral long control region (LCR). E4 seems to destabilize the cytokeratin network; E5 mediates mitogenic signals by activating the mitogen-activated protein (MAP) kinase and via this signalling pathway probably stimulates the expression of E6 and E7. E5, E6 and E7 possess proliferation-stimulating activity. Infection with papilloma virus requires the availability of epithelial cells that are still able to proliferate (basal layer cells).[35] In these cells, viral gene expression is largely suppressed, although limited expression of E5, E6 and E7 results in enhanced proliferation of the infected cells and their lateral expansion. Following entry into the suprabasal layers, 'late' viral gene expression is initiated: the circular viral genome is then replicated and structural proteins form. In the upper layers of the epidermis or mucosa, complete viral particles are assembled and released, frequently continuously for prolonged periods of time, thus contributing to successful spreading of these infections in human populations around the world.[11,35] When HPV-infected lesions progress to cervical cancer, the episomal viral DNA frequently becomes integrated in the host-cell DNA. The ring molecule is most often opened within the E2 open reading frame, disrupting the continuity of that gene. Part of E2 and adjacent open reading frames –E4, E5 and part of L2- are regularly deleted after integration.[36] E5 has recently been shown to prevent apoptosis following DNA damage.[37] However, since the E5 coding sequence is often deleted after integra

tion, E5 does not seem obligatory in late events of HPV-mediated carcinogenesis. E6 and E7 and (the overproduction of) their respective proteins play a significant role in malignant transformation, as these viral genomic parts are consistently expressed in malignant tissue and inhibition of their expression blocks the malignant phenotype of cervical cancer cells. Independently they are able to immortalize various human cell types in tissue culture, but efficiency is increased when they are expressed together.[38,39] Several functions have been described for E6 and E7. E6 protein interacts with p53 and E7 with the retinoblastoma (RB) gene product, thus blocking the activity of these tumor suppressors.[40,41] The degradation of p53, and the pro-apoptotic protein BAK -a protein involved in signalling apoptosis-, results in resistance to apoptosis of DNA damaged cells and an increase in chromosomal instability.[42] In addition, the activation of telomerase[43] and activation of SRC-family kinases, by inhibition of degradation, by the E6 oncoprotein [44] fulfil important functions in growth stimulation. The cyclin-dependent kinase inhibitor INK4A (p16) seems to counteract these functions.[45]
E7 interacts with and degrades pRB, which releases the transcription factor E2F from pRB inhibition and up regulates INK4A.[44,45] The enhanced E2F activity reduces the INK4A induced growth suppression by bypassing one of its mediators, cyclin D-CDK4, resulting in enhanced proliferation.[46] Moreover, E7 also directly stimulates the S-phase genes cyclin A and cyclin E, and seems to block the function of the cyclin-dependent kinase inhibitors WAF1 (CIP1/p21) and KIP1 (p27).[47,50] By inducing centriole amplification, E7 also induces aneuploidy of the E7-expressing cells, which contributes to tumorigenesis.[51] As mentioned above, independently E6 and E7 can immortalize human cells, but their joint function results in a marked increase in transforming activity, as there seems to be a synergistic effect. E6 seems to be impaired by INK4A, whereas E7 bypasses this inhibition by directly activating cyclins A and E. E6 in turn prevents E7-induced apoptosis by degrading the apoptosis-inducing proteins p53 and BAK.

Genetic alterations

Although the persistent presence of oncogenic HPV types and viral integration in the host DNA importantly contributes to chromosomal abnormalities, the infection with HPV alone is not sufficient for the development of cervical carcinoma. The long latency period between the onset of infection and tumor occurrence in vivo (about 13 years), combined with the fact that only part of women infected proceed to develop malignancies, indicate that other factors are involved in progression from an infected cell to a transformed cell with invasive potential.[22] In this context a combination of genetic alterations are thought to play a role. These might involve inactivation of tumor suppressor genes or activation or mutations in oncogenes.

Both copies of a tumor suppressor gene have to be inactivated in order to play a role in carcinogenesis. Usually, a mutation or deletion in one allele is accompanied by the complete loss of the other allele.[51] The resulting loss of heterozygosity (LOH) indicates the possible presence of a tumor suppressor gene in that region. In cervical carcinoma, LOH has been observed in several chromosomes in varying percentages: chromosome 3p (48%), 4p (40%), 4q (32%), 5p (17%), 6p (41%), 11p (28%), 11q (38%), 17p (24%) and 18q (24%).[52-56] Some of these might be an indicator of tumor progression since frequency increases in high-grade CIN lesions (17p) or higher FIGO stages (3p)[55] or correlates with worse survival (18q)[56].
(Proto-) oncogenes thought to play a role in cervical cancer are H-RAS, MDM-2, c-myc, her2/neu and epidermal growth factor-receptor (EGF-R). They are involved in cell replication or growth signal transduction and become activated by a point mutation (H-RAS), amplification (erb-2/neu (6-18%);HRAS (4%);c-myc (19-90%);EGF-R (13%)), or translocation.[57-60] Of these, erb-2/neu and EGF-R most prominently correlate with worse disease outcome.
With respect to quantitative alterations, a gain of 3q was observed during development from dysplasia (8%) to invasive carcinoma (90%).[61] Micro satellite instability, the phenotype of mutations in DNA repair genes, represents a relatively small subset of the total number of cervical carcinoma cases (8%).[55]

Failure of immune response
High-risk and low-risk HPV types are widespread within all human populations; infection is commonly transmitted by sexual contact and 70-80 % of infected women with cytomorphologic normal smears clear the virus within 12 months spontaneously.[22] In the other 20 % CIN will develop, but still the majority of women with CIN can clear the infection, followed by cytological regression in about 3 months.[22,62] In the group of patients that cannot resolve the virus and proceed to develop HPV-associated (pre-) malignancies, the immune system and especially failure of the immune response is thought to play an important role. These phenomena are discussed more in detail below.

CANCER AND THE IMMUNE SYSTEM

The human immune system
The immune system consists of an innate and an acquired immune system, which interact intensively. The innate arm acts as a first line of defence and protects the natural barriers (skin, mucus, secretion of body fluids containing bactericidal components), and encompasses bactericidal enzymes, the complement system,

neutrophilic granulocytes, macrophages, and NK cells. Since pathogens have the capacity to evade the innate immune defence, the acquired immune system is needed for additional defence. This is constituted by the cellular and the humoral immune system. The humoral immune response is mediated by antibodies produced by B lymphocytes that recognize foreign antibodies. Binding of the antibody to circulating antigens facilitates clearance of the antigen from the body by other cells of the immune system like macrophages. By binding to structures present at the cell surface of the host cell the complement pathway can be effectuated. Furthermore, via antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cellular cytotoxicity (CDCC) antibodies can augment effector functions of the cellular immune response.
The activity of the T cell immune system is regulated by the interaction between antigens presented by Human Leucocyte Antigen (HLA) class I and II molecules and specific T-cell receptors (TCRs) on the surface of T lymphocytes. Antigen Presenting Cells (APC) like macrophages, Langerhans'cells, dendritic cells or B cells, have the ability to phagocytose proteins and intracellular virus particles, degrade these molecules in the endocytic route and present (viral) peptides at their cell surface by HLA classe II molecules to CD4+ T-cells. The activation of naïve T-cells to recognize foreign antigens requires additional co stimulating signals besides recognition of the MHC-peptide complex. Dendritic cells (Langerhans cells in the skin) appear to be key components in activating the T-cells; besides high levels of MHC classes I and II these cells express molecules involved in co stimulation such as CD80 and CD86. Dendritic cells transport viral proteins like HPV L1 to draining lymph nodes, a crucial step in the generation of virus specific immune responses.[63] Once activated, T-cells acquire effector function (cytotoxicity and cytokine production) and proliferate. The majority of T-cells activated in response to viral infection will have a short life-span, although few persist in the circulation as memory cells, which can reactivate on subsequent encounter with antigen.[64-66]

HPV related immune responses
Understanding of natural immunity against HPV is important for the development of immunological strategies. While in most women cervical HPV infection results in a transient infection adequately eliminated within one year, in a small proportion of individuals the failing anti-HPV immune response will lead to persistent infection with the possible outcome of cancer. Cell-mediated immune responses are believed to be important in controlling both HPV infections and HPV-related neoplasms.[67] Infiltrating T cells as well as innate effector cells such as macrophages and natural killer cells have been observed in spontaneously regressing

warts, resembling a delayed-type hypersensitivity response, and warts seen in patients who are on immunosuppressive therapy often disappear when this treatment is discontinued.[68] Indirect evidence that the adaptive cellular immune system plays a major role in the protection against HPV-induced lesions is given by the enhanced prevalence of HPV infections and increased incidence of HPV-related disorders seen in individuals with an ineffective cellular immune response, such as organ transplant recipients and human immunodeficiency virus (HIV) infected patients.[9,69-71] There is some evidence that recurrence rates of CIN are higher and that invasive cancer might progress more rapidly in HIV-positive patients, both phenomena being associated with a decreased number of CD4+ T-lymphocytes.[72-74] Both T helper and cytotoxic T lymphocytes are believed to be involved in the immune reaction against HPV infections and induced disease. The presence of circulating (memory) Th cells for HPV16 E2 and E6 suggests a role in the protection against persistent HPV infection and associated development of malignancies.[74] However, T helper responses against HPV16 E7 have been associated with persistence and progression of HPV16-positive lesions[75,76] as well as with clearance of infection and regression of CIN.[77] In the case of cytotoxic T lymphocytes (CTL), contradictionary responses against HPV16 derived oncoproteins have been observed as well, since CTL against E6 and E7 were found in persistent CIN and carcinoma (in contrast to healthy individuals),[78,79] but lack of E6-specific CTLs also has been correlated to persistence of HPV16.[80] Differences in study design and study groups (healthy versus advanced stage cancer patients) may account for a great part of the inconsistencies observed between these studies.

The available data on the role of humoral immunity are not consistent too. HPVs, unlike many other human viral pathogens, do not naturally provoke a strong serological response. In transient HPV 16 infections, antibodies specific for HPV16 L1 major capsid protein, the most readily detectable virus specific immune response, only develop four months to five years after the first infection and are only measurable in one-half to two-thirds of women.[81] Antibodies specific for the E7 non-structural protein appear only with the onset of invasive cervical cancer, which however might be the logical result of the relative late expression of E6 and E7 proteins in high levels (not until in more advanced lesions).[65,82] Systemic IgG responses against L1 and L2 containing virus like particles (VLP) have been related to persistent HPV infection and development of high-grade lesions, while systemic IgA responses on the other hand have been correlated with virus clearance, although the latter responses were suggested to be a by-product of a successful cellular immune response induced at the local lymph nodes, mediated by cytokines.[83] In the cervical mucosa local IgA, more than IgG antibodies directed against HPV capsid protein, reflect a current infection.[84] On the other hand, exper-

iments performed with purified papillomavirus structural proteins –that spontaneously assemble into virus-like particles (VLPs)- of animal papillomaviruses (such as canine oral papillomavirus and cottontail rabbit papillomavirus) have resulted in effective protection against the primary infection of dogs and rabbits, respectively.[85,86] In humans, two recent randomised placebo-controlled studies on young sexually active women have shown that vaccination with HPV 16 VLPs induces absolute protection over 12-18 months against persistent infection with HPV16. In association with that, high and persisting titres of serum IgG antibodies specific for the virus were found, and similar protection occurred against virus-associated premalignant lesions of the cervix.[87,88] This suggests that, although not naturally arising, a strong induced response based on neutralizing antibodies against HPV may protect against infection.

Escape of immunosurveillance

Failure of the host immune system to control HPV infections may contribute to virus persistence and the concomitant development and progression of (pre-) malignant lesions.[64] Many intracellular, as well as intercellular, control mechanisms are operational in recognition and eradication of malignantly transformed cells by the immune system, which may prevent tumor development.[89] Escape from immunosurveillance by the tumor cells is therefore believed to be an important step during tumor development. Different mechanisms are proposed to achieve immune evasion.[64]

Due to the non-lytical viral life cycle, absence of significant inflammation and restricted expression of early proteins in the basal epithelial cell layers, HPV is limited detectable and accessible for the immune system. Papillomaviruses uptake in Langerhans cells, a subset of dendritic cells found in the epithelium of all mucosa, does not result in efficient cross presentation and activation of T cells, in contrast to dendritic cells which up regulate activation markers and secrete IL-12 after internalisation of HPV VLPs.[90] Together this may explain why it takes some women infected with HPV years to clear the virus. Furthermore, HPV interferes with interferon responses. IFNα and IFNβ are generally induced by viral infections. HPV E6 and E7 proteins are capable of blocking the production and responsiveness of infected cells to type 1 interferons (IFNs), which affects antiviral, antitumor and immunoregulatory effects.[91,92]

Another mechanism proposed in which HPV positive carcinoma cells might avoid a positive adaptive immune response is by inducing (peripheral) tolerance. E7-loaded DCs fail to mature, and immature DCs (not expressing co-stimulatory activation markers) transmit a tolerogenic rather than immunogenic signal to T cells bearing E7-directed TCRs in draining lymph nodes.[54] Chronic expression of E7 in

transformed cervical epithelial cells might serve to functionally tolerize E7-specific CD8+ precursor T cells in the T-cell repertoire, a process that has been demonstrated to occur within 2 weeks of exposure to E7 in epithelium.[93] Indeed, patients with HPV16-associated cervical carcinoma make poor E7-directed CTL responses to either endogenously expressed E7 or following E7 immunization.[78,94]
Down regulation of HLA class I molecules and up regulation of HLA class-II molecules are well established in cervical neoplastic lesions.[95,96] Certain HLA class II alleles (DRB1*13 and/or DQB1*0603. have consistently been found to be protective against the development of cervical carcinoma.[95,96] Dysregulated expression of MHC antigens precludes presentation of tumor antigen to T cells and might result in a less effective cytotoxic T-cell and Natural Killer (NK) cell anti-tumor response.[97-99]
An altered cytokine balance, systemically as well as locally at the tumor site, might achieve another potential mechanism of immune evasion. Both tumor and immune cells might thus contribute to a less effective immune response against HPV-infected, transformed cervical keratinocytes. This is discussed more in detail in the next paragraph.

Cytokines
The expression of cytokines and growth factors has been closely linked to immune function in spontaneous regression of cancer.[100] Cell-mediated immunity is regulated by cytokines, secreted by T cells, normal and malignant epithelial cells, macrophages, granulocytes and natural killer (NK) cells.[101-105] Cytokines are multifunctional and pleiotrophic and different types are distinguished based on their different functions. Type 1 cytokines are proinflammatory or immune-stimulatory and boost the cellular immune response, whereas type 2 cytokines predominantly stimulate humoral immunity and are immune-inhibitory towards the cellular immune response.[106] Pro-inflammatory cytokines are for example IL-2, IL-12, IL-15, GM-CSF, TNF-α and IFN-γ, while cytokines with strong anti-inflammatory properties are e.g. IL-10 and TGF-β_1. Some cytokines function more like autocrine or paracrine growth factors, like IL-1 and IL-6, or have pro-inflammatory as well as growth inhibitory properties (IFN-γ, IL-4 and TNF-α, even more when they are combined).[107] Cytokines can have a growth inhibitory effect on normal epithelial cells, while they can exert the exact opposite function on a malignantly transformed specimen of the same lineage.[108] They can interfere with cancer cell motility and invasion via up regulating of E-cadherin cell surface adhesion molecules (IL-12), while IL-6 does the opposite.[109,110] Type 1 cytokines exert potent antitumor effects, as summarized by the following observations.
First) IL-12 is a potent activator of cellular immunity, has anti-tumor and anti-

metastatic activities against several murine tumors and up-regulates IFN-γ production;[111,112] second) IL-2, IL-12 and IFN-γ activate CTL- and NK-mediated cytolytic functions associated with effective anti-tumor defence mechanisms;[113] third) IL-2 induces the transformation of NK cells into lymphokine-activated killer (LAK) cells;[112] fourth) IL-12 and IFN-γ inhibit angiogenesis induced *in vivo* by human tumor cell lines;[114] fifth) IFN-γ enhances the presentation of antigenic peptides to T helper lymphocytes and directly inhibits the growth of cervical carcinoma cell lines.[115,116]

In contrast, type 2 cytokines, in particular IL-10 and TGF-β_1, were shown to down-regulate tumor-specific immune responses;

First) by directly suppressing IFN-γ and IL-12 production, thereby preventing the activation of CTLs and NK cells, as well as that of LAK cells;[107,117,118] second) by reducing HLA expression on the surface of tumor cells, thus preventing the optimal expression of binary complexes formed by tumor antigen in association with HLA molecules on the surface of such cells;[17,119] and third) by inhibiting tumor antigen presentation by and cytotoxic activity of antigen-presenting cells.[120]

Production of cytokines by immune cells

It has been realized that qualitative as well as quantitative alterations in cytokine production can result in complex and severe impairments of immune function.[121] In HIV infected individuals a decline in type 1 cytokines in combination with an increase in type 2 cytokines was observed to be associated with, and predictive for, progression of HIV infection to acquired immune deficiency syndrome (AIDS).[122-124] A similar decrease in the Th1 response with predominance of the Th2 cytokine profile has also been observed in patients with neoplasms such as Sezary syndrome, Hodgkin's disease, bronchogenic carcinoma, renal cell carcinoma, lymphomas, glioma, basal and squamous cell carcinoma and melanoma.[125-135] In patients and mice successfully treated and in remission of disease the inverse switch from Th2 towards Th1 pattern was observed.[126,137]

Healthy HPV-16 negative women have been shown to demonstrate a mixed Th1/Th2 cytokine profile following antigenic stimulation with HPV-16 peptides, suggesting that naturally arising virus-induced immunity requires both responses.[138] In patients with HPV-related disease, changes in systemic cytokine production pattern of peripheral blood mononuclear cells (PBMC) are believed to be of importance in the development of (pre)malignant lesions too.[106,138-141] Besides, locally at the tumor site, disturbance of the cytokine balance may also favor immune escape and tumor growth. Few studies suggest that the transformation zone in itself, where the majority of dysplasias originate, is a locus resistentiae minoris because of enhanced IL-10 production in Langerhans cells and lymphocytes in compari

son to similar cells in the (normal) ectocervix.[142-144] Tartour *et al.* showed, that the presence of lower levels of intratumoral IFN-γ in tumor biopsies, was related to a worse survival rate and higher disease recurrence; a significant relationship between lower intratumoral IFN-γ and absent HLA class II expression was found in this group of invasive cervical cancer.[120] A similar decrease in type 1 cytokine production in (pre)malignant tissue compared to normal cervical epithelium was detected by others,[105,145-147] whereas also an increase in type 2 cytokines in this tissue has been reported.[143,148-151]

Production of cytokines by tumor cells

Most of the above studies focus on cytokine expression by PBMC, (subepithelial) immune cells or investigate total cytokine mRNA expression from tumor biopsies, through by which the separate contribution of inflammatory cells and tumor cells cannot be distinguished. There are however many indications, that (pre-) malignant epithelial cells contribute to local immune suppression by the production of immunomodulatory cytokines and chemokines too.[146,152-159] Cervical carcinomas, as most other solid tumors, contain many lymphocytes as well as leucocytes such as macrophages, eosinophils, neutrophils, dendritic cells and mast cells, both in the supporting stroma and among the tumor cells, which conventionally are assumed to reflect the host defence mechanism against the tumor cells.[160,161] The conventional idea about the macrophage component is, that they are recruited to the tumor site by tumor cell derived monocyte chemotactie protein-1 (MCP-1/CCL2), macrophage colony stimulating factor (CSF-1 or M-CSF) and vascular endothelial growth factor (VEGF) and, following activation by IL-2, interferon and IL-12, may kill neoplastic cells.[162] However, the local cytokine milieu present in many tumors tends to block the immunological functions of the macrophages such as antigen presentation and cytotoxicity, and diverts them towards an immunosuppressed and trophic phenotype, by especially the production of IL-6 and CSF-1.[163] IL-4 and IL-13 can reverse these effects, while GM-CSF and IFN-γ direct tumor associated macrophages (TAM) towards a more cytotoxic and antigen-presenting phenotype too. Besides educating the TAM in such a way that they become immunologic neutral, regulatory and pro-tumorigenic, tumor cells might also modulate the lymphocytes in the tumor infiltrate. Tumor cell derived IL-10 and TGFβ$_1$ has been shown to direct tumor infiltrating lymphocytes in cervical carcinoma towards a Th2/Tc2 polarity.[152] Furthermore, by the production of inflammatory cytokines and chemokines such as MCP-1, IL-8 (CXCL8) and eotaxin (CCL11), tumor cells are believed to attract preferentially polarized Th2 cells and T regulatory (Tr1) cells to the tumor site.[164] The Tr1 cells in turn, may prolong tumor cell persistence by suppressing protective Th1 responses via a fur

ther secretion of immunosuppressive cytokines such as IL-10 and TGF-β.[155-167] Although it may seem paradoxically, inflammation is indispensable for tumor invasion and metastasis; limited inflammation results in restricted vascularization and restricted tumor growth, while abundant pro-inflammatory chemokines result in inflammation, neovascularization and rapid tumor growth.[168,169] Studies in different types of cancer have indeed demonstrated a correlation between an abundance of TAM and tumor progression and/or poor prognosis, as have been shown for over expression of macrophage growth factors and chemokines and poor survival,[150,162,163,170,171] suggesting the need for inflammatory cells for tumor progression.

Thus, the tumor infiltrate accompanying most cervical carcinomas may on the one hand represent the host defence mechanism to attempt to eradicate HPV infected and malignantly transformed cells, but may, on the other hand, stimulate tumor growth due to a potentially immunosuppressive cytokine and chemokine network in the tumor. The balance in the cytokine network of the progressing tumor probably pushes the inflammatory cells in the direction of pro-tumor or anti-tumor activity.

TUMOR STROMA, ECM AND MODULATING FACTORS IN CANCER

The tumor stroma is a specialized stroma that accompanies carcinomas and is characterized by modifications in the non-epithelial cell types that secrete extra cellulair matrix (ECM) proteins and growth factors. Besides glycoproteins such as fibronectin, laminin, tenascin, vitronectin and collagen, the tumor stroma contains (activated) fibroblasts, inflammatory cells and cells comprising the vasculature (endothelial cells, pericytes and smooth muscle cells). It has been long recognized that carcinomas induce a modified stroma through the expression of growth factors that promote angiogenesis, altered ECM expression, accelerated fibroblast proliferation and increase inflammatory cell recruitment.[172] This stroma is thought to be indispensable for the tumor to grow and metastasise, since one of its functions is to provide the vascular supply the tumor requires for nourishment and gas exchange. Impaired interactions of epithelial cells with ECM can result in the transformation of the epithelia into carcinoma. Fibroblasts are responsible for the synthesis, deposition and remodelling of much of the ECM in the tumor stroma and they are an important source of paracrine growth factors that support survival and proliferation of carcinoma cells. Probably, a cross talk occurs between carcinoma cells and cells in the tumor stroma by producing a scala of auto- and paracrine factors (cytokines, growth factors, proteases and integrins),

which cooperation might influence the biological behaviour of the tumor and might have clinical consequences too.[173] Some of these factors, in which TGF-β_1 plays a central role, are discussed below.

TGF-β_1

TGF-β_1 is one of the most potent anti-inflammatory cytokines.[107,117,118] As described above it can exert different immunosuppressive functions on several cells of the immune system. However, as a member of a superfamily of growth factors, this cytokine displays many more functions that might be advantageous for tumor growth. Practically every cell in the body is capable of producing TGF-β_1 and has receptors for this molecule. It is secreted as part of an inactive, small latent complex (SLC) and cleavage of active TGF-β_1 from this latent complex can be accomplished by proteolytic enzymes (such as plasmin), thrombospondin, serine proteinases, matrix metallo proteinases and integrins expressed by tumor cells,[171,174] of which the latter will be described more detailed in the $\alpha v\beta 6$ integrin paragraph. After binding of active TGF-β_1 to the TGF-β transmembrane receptor II (TβRII), TβRI is recruited and phosphorylation of serine and threonine residues of TβRI by TβRII leads to activation of the kinase. Subsequently the signal is propagated inside the cell through phosphorylation of the intracellular signal transducers SMAD 2 and SMAD 3, which form complexes with SMAD 4. These complexes accumulate into the nucleus, where they control gene expression through interaction with transcription factors.[175] The inhibitory SMAD 6 and SMAD 7 can antagonize signalling by either competitive binding with TβRI or dephosphorylating this receptor.

In non-malignant cells TGF-β_1 displays growth inhibitory activities via a reversible G1 arrest in the cell cycle and thus might act as a tumor suppressor in early stages.[176] However, during carcinogenesis tumor cells can lose responsiveness to TGF-β_1, resulting in a proliferative advantage over TGF-β_1 sensitive cells.[177] Among the possible mechanisms by which tumor cells lose responsiveness to TGF-β_1 are inactivation, mutation or loss of TGF-β receptors on the tumor cells, inability to activate the latent form of TGF-β_1 and loss of functional intracellular signalling pathways.[178-184]

When the cellular response of the tumor to TGF-β_1 is compromised, TGF-β_1 production can be beneficial for tumor growth because of proliferative advantage over other cells and suppression of immunosurveillance at the tumor site. Other pathways by which production of TGF-β_1 by tumor cells can augment tumor growth are promotion of angiogenesis, resulting in an increased blood supply to the tumor cells, and promotion of metastasis formation.[185-187] Furthermore, TGF-β_1 regulates the formation of stroma and deposition of extra cellular matrix (ECM)

by stimulating fibroblasts and other cells to produce ECM proteins such as collagens, fibronectin, vitronectin, laminin and proteoglycans, while concomitantly down-regulating the expression of ECM-degrading proteinases and up regulating proteinase inhibitors like plasminogen activator inhibitor (PAI-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1).[188-192] Taken the above in account it is not surprising that the enhanced presence of TGF-β_1 or the disturbed signalling of it, by which tumor cells lose their responsiveness to growth inhibition, in many different cancers is associated with poor patients disease free survival.[85,193-197]
Data on the role of TGF-β_1 in cervical cancer are conflicting; enhanced pre-treatment plasma levels have been reported to reflect tumor burden and predict shorter survival rates,[198,199] while on the other hand TGF-β_1 levels in serum have been demonstrated to be lower in patients with invasive disease compared to healthy persons or CIN patients.[200,201] However, at least there seems to be an important role for TGF-β_1 during early carcinogenesis, as the expression in tissue is often observed to diminish from normal epithelium via CIN 1-3 to invasive cancer.[202-204] This quantitative decrease in TGF-β_1 expression may result in an increasing loss of TGF-β_1 mediated growth inhibition during malignant transformation, while additionally HPV E7 is thought to interfere with the cell's sensitivity to TGF-β_1-mediated growth inhibition by disturbing the intracellular signalling pathway by blocking binding to SMAD 3.[205,206]
Although (part of the) malignantly transformed cervical cells might lose their sensitivity to TGF-β-induced growth arrest, others have demonstrated that other TGF-β transcriptional responses, such as the induction of PAI-1, are maintained.[207,208]

PAI-1

The plasminogen activator (PA)-plasmin proteolytic system has been implicated in the processes of tumor cell invasion and metastasis via the coordination and regulation of a series of adhesive, proteolytic and migratory events. Urokinase-type (u-PA) and tissue-type (t-PA) plasminogen activators are serine proteinases that catalyse the conversion of inactive plasminogen into plasmin, a broadly acting enzyme able to degrade a variety of ECM proteins and to activate matrix metalloproteinases and growth factors. Plasminogen and uPA bind to their specific receptors (uPARs) directing plasmin activity to the migrating tumor cell surface.[209-212] The activities of PA are directly controlled by specific serine protease inhibitors (serpins), the PA inhibitors 1 and 2 (PAI-1 and –2). It has been generally assumed that proteases are necessary to degrade the basement membrane and the ECM to permit the penetration by tumor cells of surrounding tissues and blood vessels and of endothelials cells to neovascularize.[213] Many studies have indeed demonstrated a correlation between uPA expression and cellular invasion and

metastasis as well as reduction of metastatic potential by using neutralizing antibodies or antisense oligonucleotides.[214,215]

However, a paradoxical association between poor prognosis in patients with cancer and high levels of expression of protease inhibitors like TIMP-2 and PAI-1 has been reported for a variety of cancer types[216-232]. Previously, it has been postulated that peritumoral PAI-1 production in stromal cells serves as a defence mechanism against tissue destruction by tumor cell proteolysis[233]. However, the conclusion that in the PAI-1 deficient mice an increased invasion and tissue destruction would be present was not demonstrated[234]. Other investigators have shown that PAI-1 competitively binds with u-PAR to the ECM component vitronectin and that by inhibiting cell adhesion to vitronectin PAI-1 promotes endothelial cell migration from vitronectin to fibronectin[225,226]. Although not completely elucidating the paradoxical activity of PAI-1, these data highlight the importance of a balance between proteases and their inhibitors during tumor angiogenesis.

Another factor of importance might be the cooperation between PAI-1 and TGF-β_1, as this cytokine is known to induce PAI-1 expression *in vitro* and *in vivo*[191,227-231]. As described above, both factors are involved in ECM modelling, stroma formation and angiogenesis; by up regulating PAI-1, TGF-β_1 might reinforce these effects.

To be able to perform its different activities, TGF-β_1 has to be cleaved from the small latent complex, after which it becomes active, a process that can be accomplished by several factors among which these specific integrins.

$\alpha v\beta 6$ integrin

Integrins are transmembrane cell surface receptors, composed of non-covalently linked heterodimers of α and β chains, both of which contain a large extracellular domain and a short, COOH-terminal cytoplasmic domain[235]. At least 20 different integrins are known to interact with a variety of ECM components and some of these are known to mediate cell-cell adhesion by binding to membrane-proteins such as ICAMs or VCAMs[233]. In addition, the cytoplasmic tails of integrins transduce signals by associating with adaptor proteins that connect the integrin, cytoskeleton, cytoplasmic kinases and transmembrane growth factor receptors, thus coordinating signaling pathways that regulate a diverse range of cell functions.[234]

The αv integrins form a subfamily of five members ($\alpha v\beta 1$, $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha v\beta 6$ and $\alpha v\beta 8$) that recognize a group of overlapping ligands that generally contain the canonical tripeptide recognition sequence, arginine-glycine-aspartic acid (RGD). Within these, the $\alpha v\beta 6$ integrin binds to RGD sites in its ligand proteins fibronectin, latency associated protein (LAP) and to a lesser extent vitronectin

and tenascin.[235] αvβ6 is predominantly expressed on epithelial cells, but down regulated on differentiated epithelia. However, in fetal development, wound-healing and a variety of carcinomas high or *de novo* expression of this integrin is detected.[233] The αvβ6 integrin has been described to bind and activate latent TGF-β_1.[174] Latency associated protein (LAP) and TGF-β remain noncovalently associated, in which configuration TGF-β is unable to bind to its receptors (latent TGF-β). In most cases, the complex of LAP and TGF-β (the small latent complex, SLC) is joined by latent TGF-β binding protein 1 (LTBP1); latent TGF-β can be linked by LTBP to binding sites in the extracellular matrix.[172] αvβ6 has a high affinity for the RGD sequence in the LAP of TGF-β_1 and TGF-β_3; after binding, this complex is tethered by a disulfide linkage to LTBP1, which is essential for TGF-β activation. A range of as yet unspecified extra cellular signals lead to tight association of the integrin with the actin cytoskeleton and induction of a conformational change in the latent complex, which results in presentation of the active site of TGF-β to the receptor and the subsequent signaling cascade.[215]

Vice versa, in wound healing studies and in carcinomas, TGF-β_1 has been demonstrated to up regulate the expression of αvβ6 integrin on keratinocytes and colon carcinoma cells, respectively.[236-238] Up regulation of αvβ6 on these cells resulted in an enhanced potential to migrate on fibronectin-coated transwells, which could be inhibited by a function-blocking β6 antibody. In this fashion TGF-β_1 is thought to cause epithelial-mesenchymal-transition (EMT), a process required for tumor cell invasion and metastasis, by up regulating the integrin αvβ6 on epithelial cells.[238,239]

Furthermore, αvβ6 may have multiple other regulatory functions in oncologic processes. Enhanced or *de novo* expression has been observed in different epithelial malignancies such as oral squamous, breast, colon, gastric and ovarian carcinoma and over expression of αvβ6 in some cancers has been shown to be associated with unfavourable clinical parameters and decreased survival.[234,238,240-245] Whereas the above described processes are mediated by the extra cellular or transmembrane domain of the integrin, the cytoplasmic domain affects tumor proliferation, uPA and MMP-2 and MMP-9 production, MAP kinase activation, migration and apoptosis upon binding to ligand.[234,242,245-248]

IMMUNE STRATEGIES IN HPV-ASSOCIATED CERVICAL NEOPLASMS

The currently used immunological therapeutical strategies in cervical cancer consist mainly of vaccines. Prophylactic vaccination with virus-like particles (VLPs) has been demonstrated to induce protection of humans and animals against per

sistent HPV 16 infection (and thus in future possibly against cervical cancer).[85-88] However, since protection against HPV-associated diseases after VLP vaccination is genotype specific and the prophylactic vaccines that are envisaged presently incorporate two high risk genotypes, HPV 16 and 18, at best only two thirds of cervical cancers in successfully immunized women can be prevented.[249,250] According to epidemiological evaluation of geographic variation in HPV types, a polyvalent vaccine including the 7 most common HPV types (16, 18, 45, 31, 33, 52 and 58. would prevent about 87% of cervical cancers worldwide.[250]
Therapeutic vaccines should induce specific cell-mediated immunity that prevents the development of lesions and eliminates preexisting lesions or malignant tumors. Most vaccines described in experimental systems targeting HPV-16 E6 and E7 proteins have been shown to generate strong CD4 and CD8-dependent CTL activity and anti-tumor responses in murine tumor systems or preclinical studies,[251-258] as have methods such as the transduction of tumor cells with genes encoding costimulatory molecules or cytokines[259-261] or adoptive transfer of CTL raised against a HPV 16 E7 epitope.[262-264] However, clinical trials for cervical cancer interventions are hampered by the fact that they are carried out in patients with late-stage disease, who often are systemically immunocompromised by radiotherapy and/or chemotherapy.[265] Additionally, the tumor cytokine network might establish further local immune suppression. This indicates that an effect of therapeutic vaccines can only be expected in at least partially immunocompetent patients and that the local tumor environment might need to be modulated too.

SCOPE AND OUTLINE OF THE THESIS

The goal of our study was to gain further inside in the mechanisms by which tumor cells might be able to modulate the microenvironment at the tumor site in relation to the host immunological response. For this purpose, the paracrine effects of cervical carcinoma cell derived factors such as different cytokines, growth factors and integrins were investigated. A better understanding of these mechanisms might be beneficial both in the adjustment of conventional therapy as in the development of immunotherapeutical strategies.
Chapter 2 describes the profile of cytokines produced by both normal cervical keratinocytes and cervical carcinoma cell lines and the possible influences of changed cytokine production due to malignant transformation on the host immune response. Pro- and anti-inflammatory cytokines, growth- and chemotactic factors were investigated. Subsequently, because TGFβ_1 was abundantly produced in cervical carcinoma cell lines and has been demonstrated to have strong

immuno-inhibitory qualities as well as stimulatory properties on stroma formation and angiogenesis, in **Chapter 3** the effects of this multifunctional cytokine on the tumor infiltrate as well as the formation and composition of the tumor stroma are investigated. For this purpose TGFβ$_1$ mRNA expression in carcinoma cells on tumor slides was investigated by RNA *in situ* hybridisation on a large series of predominantly squamous cell carcinomas. This material came from surgically treated patients of whom clinical data were gathered in a prospective databank. **Chapter 4** focusses on the relationship between TGFβ$_1$-mRNA and PAI-1 protein, which is induced by active TGFβ$_1$, and clinical and histopathological disease parameters. The effects of both molecules on the development of clinical disease and their prognostic relevance in cervical cancer are discussed.
To be able to perform its diverse activities, TGFβ$_1$ needs to be cleaved from the latent complex to which it remains bound in inactive state. One of the proteins capable of achieving this belongs to the subfamily of αv integrins. For this purpose we describe in **Chapter 5** the role of one of these integrins in cervical cancer. The αvβ6 integrin has high affinity for latency associated protein, to which TGFβ$_1$ is bound, and for fibronectin, both present in the ECM of cervical carcinomas, and is a potent activator of latent TGFβ$_1$. In several carcinomas αvβ6 expression is associated with an enhanced potential of the tumor cells to invade the surrounding tissues and metastasise. TGFβ$_1$ on the other hand, has been demonstrated to up regulate the expression of several integrins (including αvβ6) on keratinocytes in wound healing. Besides in primary carcinomas, expression of αvβ6 in CIN and lymph node metastases was studied to investigate its role in cervical carcinogenesis. Finally, in **Chapter 6** the studies presented are summarized and the relevance with respect to cervical cancer and future perspectives are discussed.

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CHAPTER 2