Transforming Growth Factor beta-1 in cervical cancer

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TRANSFORMING GROWTH FACTOR β_1 INDUCES TUMOR STROMA AND REDUCES TUMOR INFILTRATE IN CERVICAL CANCER

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Abstract

Objective: cervical carcinomas consist of tumor cell nests surrounded by varying amounts of intratumoral stroma containing different quantities and types of immune cells. Besides controlling (epithelial) cell growth, the multifunctional cytokine Transforming Growth Factor β_1 (TGF-β_1) is involved in formation of stroma and extracellular matrix (ECM) and in immunosuppression. Several malignancies are known to be associated with enhanced TGF-β_1 production, repression or mutation of TGF-β transmembrane receptors or mutations at the post-receptor intracellular signaling pathway. The aim of our study was to investigate the role of tumor cell derived TGF-β_1 on the amount of intratumoral stroma, the deposition of collagen IV, fibronectin and laminin, and the tumor infiltrate in cervical carcinoma.

Methods: the expression of TGF-β_1 mRNA in 108 paraffin embedded cervical carcinomas was detected by mRNA *in situ* hybridization. Immunohistochemistry was used to investigate the amount of tumor stroma and ECM-proteins and the extent of the tumor infiltrate. Plasminogen activator inhibitor-1 (PAI-1) protein expression in tumor cells was determined to verify the biological activity of TGF-β_1.

Results: cytoplasmatic TGF-β_1 mRNA expression in tumor cells was significantly correlated with the amount of intratumoral stroma and the deposition of collagen IV. TGF-β_1 mRNA expression in every tumor was accompanied by PAI-1 expression, indicating biological activity of TGF-β_1. An inverse relationship between TGF-β_1 mRNA expression in tumor cells and the extent of the tumor infiltrate was demonstrated.

Conclusions: our results indicate that cervical cancer cells affect the amount and the composition of the intratumoral stroma and the tumor infiltrate by the production and secretion of TGF-β_1

Introduction

Cervical cancer is the second leading cause of cancer death in women worldwide.[1] Bearing in mind the important role that chronic infection of keratinocytes with human papilloma viruses (HPV) plays in the pathogenesis of cervical cancer, the host cellular immune response is thought to be essential in controlling both HPV infections and HPV-related neoplasms.[2-6] Histologically, cervical carcinomas show epithelial tumor cells growing in tumor cell nests, surrounded by stroma. The wide variety in the amount of the stromal component results in a difference of cancer growth pattern ranging from diffuse to cohesive.[7-9] The tumor stroma provides the vascular supply that tumors require for nourishment, gas exchange and waste disposal, and it may also limit the influx of inflammatory cells, thus providing a barrier for immunologic rejection.[10] The process of tumor invasion and metastasis requires complex changes in the normal epithelial cell-cell and epithelial cell-stroma interactions. In addition to cellular adhesion molecules, extracellular glycoproteins like fibronectin, laminin or tenascin may be involved in the complex biological cascade of cancer invasion and metastasis.[7,9,11]

The capacity of cervical cancer cell lines and cervical cancer cells ex vivo to produce and secrete TGF-β_1 is well known.[12-14] TGF-β_1, a member of a super family of growth factors, plays an important role in the regulation of various physiologic cell processes. Practically every cell in the body produces TGF-β_1 and has receptors for this molecule. TGF-β_1 is a multifunctional and pleiotropic cytokine. TGF-β_1 interferes in most cells with proliferation by displaying a growth inhibitory activity via a reversible G1 arrest.[15] Furthermore, TGF-β_1 regulates the formation of stroma and deposition of ECM by stimulating fibroblasts and other cells to produce ECM proteins such as collagens, fibronectin, vitronectin, laminin and proteoglycans.[16,17] Concomitantly, TGF- β_1 down-regulates the expression of ECM-degrading proteases and induces proteinase inhibitors like PAI-1 and tissue inhibitor of metalloproteinase-1 (TIMP-1). TGF- β_1 also promotes fibrotic reactions, probably a combined effect of stimulation of fibroblast chemotaxis, inhibition of epithelial regeneration and induction of ECM synthesis.[11] Another major biological effect of TGF-β_1, immunosuppression, contains its ability to inhibit the generation of cytotoxic T lymphocytes (CTLs) and to inhibit the production of the immunostimulatory cytokines IFN-γ and TNF-α by T lymphocytes.[18] Furthermore, antigen presentation by macrophages and maturation of dendritic Langerhans'cells can be blocked by TGF-β_1.[15]

Tumor cells can be an important source of TGF-β_1. Several different malignancies, like gastric, colorectal, ovarian, prostatic and pancreatic cancer, have been associated with enhanced TGF-β_1 production or with alterations in the signalling

pathway, such as disturbed TGF-β receptor or SMAD expression.[19-23] Some studies propose mutations in or downregulation of expression of TGF-β receptors I and II (*TGFβR-I and –II*) on cervical carcinoma cells as a possible explanation for resistance to growth inhibition by TGF-$\beta_{1,}$ thus resulting in a growth advantage of tumor cells over other cells.[24-27] However, for the greater part this was investigated in carcinoma cell lines. Observing the diversity in growth pattern of cervical carcinomas, we were intrigued by the question if these patterns could be explained by a paracrine effect of TGF-β_1 produced by tumor cells. Therefore, we analyzed the correlation between TGF-β_1 mRNA expression by carcinoma cells and percentage of intratumoral stroma. Also the amount of deposition of the ECM proteins fibronectin, collagen IV and laminin in stroma was measured. In addition, the extent of the tumor infiltrate within the tumor stroma was investigated. Since expression of the PAI-1 gene is strongly induced by TGF-β_1 and often used as a marker for TGF-β-induced transcription *in vitro*,[28-33] we examined expression of the PAI-1 protein in tumor cells. Although PAI-1 expression *in vivo* might be affected by multiple factors, TGF-β_1 expression has been put in connection with PAI-1 production *in vivo* by more authors.[30,34-36]

Material and methods

Tissue samples

From 108 patients with carcinomas of the uterine cervix who underwent radical hysterectomy with lymphadenectomy between 1985 and 1995, formalin-fixed paraffin-embedded tissue blocks were retrieved from the archives of the Department of Pathology, Leiden University Medical Center. None of the patients had received any therapy prior to surgery. For immunohistochemistry, paraffin blocks containing a representative part of the tumor were used.

Histopathological features

Slides of all tumors were reviewed using conventional histologic sections stained with hematoxylin and eosin.[30] Tumors were classified as squamous cell carcinoma, adenocarcinoma or adenosquamous carcinoma. Periodic acid-Schiff staining with diastase pretreatment and Alcian-blue staining was used to assign tumors with mucin production and squamous morphology to the adenosquamous category.

RNA in situ hybridization

The *in situ* hybridization was performed on paraffin-embedded sections of the 108 cervical tumors and carried out as previously described.[38,39] In short, we used

a *Sma*I-*Bam*HI fragment of TGF-β_1 complementary DNA (cDNA) cloned into pBluescript KS (Stratagene, La Jolla, CA). The specific copy RNA (cRNA) probes were labeled with digoxigenin following the manufacturer's protocol (Boehringer, Mannheim, Germany). After pretreatment the tumor sections were hybridized with 10 ng TGF-β_1 antisense riboprobe per slide during 16 h at 62 ^{0}C. Subsequently, sections were washed in 2x standard saline solution citrate (SSC) with 50% formamide at 50 ^{0}C, then in 0.1x SSC with 20 mM β-mercaptoethanol at 62 ^{0}C, and finally treated with 2 U/ml ribonuclease (RNAse) T1 (Roche, Basel, Switzerland) in 2x SSC plus 1 mM ethylenediaminetetraacetic acid (EDTA) at 37 ^{0}C. The immunodetection of digoxigenin-labeled hybrids was done using nitro blue tetrazolium (NBT) as chromogen and bicholylindolyl phosphate (BCIP) as coupling agent (Roche). Blue staining of the cytoplasm indicated positivity for TGF-β_1 mRNA. Adjacent tumor slides, hybridized with TGF-β_1 sense riboprobes, were included as negative controls and in general did not show staining. Normal kidney tissue served as a positive control.

Immunohistochemistry

Immunohistochemistry was performed on 4 μm sections using aminopropylethoxysilane (APES) coated slides. Paraffin sections were deparaffinized and rehydrated, and endogenous peroxidase was quenched with 0.3 % H_2O_2 in methanol for 20 min. Antibodies used are listed in table 1. Incubations were performed at room temperature. Phosphate buffered Saline (PBS) containing 1% Bovine Serum Albumine (BSA) was used as diluent for all antibodies. Washing in between incubations was performed 3 times for 5 min each in PBS. Incubation with the antibodies against laminin, fibronectin and collagen IV was preceded by pretreatment with 0.4% pepsin in 0.01 M HCl for 20 min at 37^{0}C. After washing in PBS, slides were incubated overnight with the specific primary antibodies. Biotin-labeled rabbit anti mouse immunoglobulins and a biotinylated horseradish

Table 1 - Antibodies.

Antibody	Source	Pretreatment	Dilution	Supplier
PAI-1	mouse	none	1:500	American Diagnostics inc., Greenwich, CT, USA
Fibronectin	goat	pepsin	1:1000	Sigma, St. Louis, MI, USA
Laminin	rabbit	pepsin	1:1000	Heyl, Berlin, Germany
Collagen IV	rabbit	pepsin	1:3000	Heyl, Berlin, Germany
TGF-b RI	rabbit	none	1:75	Santa Cruz Biotechnology, Santa Cruz, CA, USA
TGF-b RII	rabbit	none	1:250	Santa Cruz Biotechnology, Santa Cruz, CA, USA

Polyclonal antibodies used for immunohistochemical experiments to investigate the histological parameters. The source they are derived from, the pretreatment method, the dilution used and the supplying companies are also listed.

peroxidase (HRP)-Streptavidin complex (both DAKO) were subsequently applied for 30 min each. To visualize immune complexes a 0.05% solution of diaminobenzidine (Sigma) containing 0.0018% H_2O_2 in a 0.05 M Tris-HCl buffer (pH 7.6) was applied. Mayer's Hematoxylin was used for counterstaining of the slides.
Brown staining of cytoplasm and/or plasmamembrane indicated positivity for *TGF*β*-RI* and *–II* and of PAI-1. Brownstaining of ECM components indicated positivity for fibronectin, laminin, collagen IV and PAI-1 in stroma. As a negative control for polyclonal antibodies, rabbit IgG on serial slides was used. Appropriate positive control sections were stained simultaneously. For *TGF*β*-RI, -II* and PAI-1 a mamma carcinoma was used and for fibronectin, laminin and collagen IV normal kidney tissue served as positive control. Specificity of *TGF*β*-RI* and *–II* was verified by absorption with immune peptide.

Immunohistochemical evaluation
Staining for TGF-β_1 mRNA, PAI-1, *TGF*β*-RI* and *-II* in tumor cells was scored semiquantitatively according to a system proposed by Ruiter *et al.*[40] Scores representing the percentage of tumor cells stained positive were as follows: 0 (no positive tumor cells); 1 (1-5%); 2 (6-25%); 3 (26-50%); 4 (51-75%); and 5 (76-100%). Intensity of tumor cell staining was scored as 0 (no staining); 1 (+, weak); 2 (++, clear); and 3 (+++, bright). A final score was calculated by adding the scores for percentage and intensity, resulting in scores of 0 to 8. A score of 0 was deemed negative; 2-4 was considered weak, 5-6 was considered moderate and 7-8 was considered strong.
Stroma within the tumor was mainly formed by fibroblasts, ECM proteins, blood vessels and some immune cells. To determine the total amount of stroma within the tumor, the number of tumor cells per tumor was counted using an ocular grid as described by Jonges *et al.* and Hagenaars *et al.*[41,42] This was done in sections stained for fibronectin and counterstained with Mayer's Hematoxylin (magnification x 100), in which discrimination between tumor cells and stromal tissue could be clearly made. For each tumor slide, tumor cells in 5 different grid-fields, each representing an area of 0.16 mm^2, were counted and the mean was calculated. The fields to count were randomly chosen but attention was paid not to confuse tumor stroma with normal (patients) stroma. We counted 5 fields, since in many tumor sections due to size, fields were overlapping when more were chosen. For each tumor the percentage of area occupied by stroma was determined by subtracting the mean amount of tumor cells from 100.

Extracellular matrix staining for the tumor stroma components fibronectin, laminin and collagen IV was scored at the tumor-stromal border as previously

described by Havenith *et al.*[43]: 1 (less than 25% immunoreactivity); 2 (between 25 and 75% immunoreactivity); and 3 (more than 75% immunoreactivity). PAI-1 staining of the tumor stroma was scored at the tumor-stromal border as sporadic, local or diffuse.[40]

On tumor tissue adjacent to the tissue on which *in situ* hybridization and immunohistochemistry were performed, the inflammatory infiltrate was assessed on HE slides at the advancing front of the tumor according to the criteria used by Jass *et al.*[44] and scored as: mild, moderate and extensive.

HPV detection and typing
All 99 samples were tested and subtypes were determined as described before.[37]

Statistical analysis
Statistical analysis was performed using the SPSS 10.0 software package. TGF-β_1 mRNA expression was correlated to intratumoral stroma percentage, ECM protein expression, amount of tumor infiltrate and PAI-1 protein expression. Correlations were evaluated with the chi-square test and the Fisher's exact test. Linear correlations were evaluated using the Anova regression model. Results were considered statistically significant if the p-value $\leq$ 0.05.

Results

Assessment of the slides
TGF-β_1 mRNA expression was examined in the cytoplasm of cervical carcinoma cells. Inflammatory cells, known to produce TGF-β_1, served as an internal control for the mRNA quality of the slide. When neither tumor cells nor inflammatory cells stained positively in a slide, it was assumed that the mRNA quality of the specific tissue was decreased, probably due to too long fixation in the formalin, resulting in exclusion of that tumor. In our study this resulted in exclusion of 9 completely negative cases on the total number of 108. In the remaining 99 tumors (84 squamous carcinomas, 9 adenocarcinomas and 6 adenosquamous carcinomas), TGF-β_1 mRNA expression was weak in 9 cases, moderate in 40 cases and strong in 50 cases. Normal cervical epithelial cells, present in the majority of the tissue slides, demonstrated moderate to strong staining, as did the inflammatory cells in the tumor infiltrate (Fig 2).

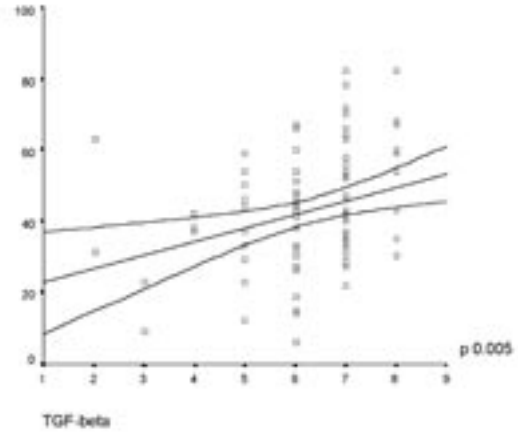

Figure 1 - Scatterplot with regression line between the TGF-β_1 mRNA expression by cervical carcinoma cells (range: 2 to 8) and the percentage of intratumoral stroma (range: 6 to 82 %, see M&M), analyzed with an analysis of variance regression model (Anova). The correlation is statistically significant (p 0.005).

PAI-1 staining of tumor cells was weak in 5 cases, moderate in 32 cases and strong in 62 cases (Table 2, Fig 3). Normal cervical epithelial cells and inflammatory cells, especially eosinophilic granulocytes, showed positive staining for PAI-1 as well. Stromal staining for PAI-1 was sporadic in 41 cases, local in 37 cases and diffuse in 21 cases (Table 2).

Table 2 - Relationship between strong TGF-β1 expression and ECM-/ PAI-1-expression and inflammatory infiltrate in 99 patients with cervical carcinoma.

		N	TGF-β1 strong n/(%)	P value
Collagen IV	<25%	92	43 (47%)	**0.03**
	25-75%	6	6 (100%)	
	>75%	1	–	
Laminin	<25%	96	48 (50%)	1.0
	25-75%	3	2 (70%)	
	>75%	–	–	
Fibronectin	<25%	20*	8 (50%)	0.22
	25-75%	28	12 (43%)	
	>75%	49	29 (59%)	
Tumor infiltrate	Minor	49	30 (61%)	**–0.04**
	Moderate/extensive	50	20 (40%)	
PAI-1 stroma	Sporadic	41	23 (56%)	0.30
	Local	37	15 (41%)	
	Diffuse	21	12 (57%)	
PAI-1 tumor	Weak/moderate	37	19 (51%)	0.79
	Strong	62	31 (50%)	

In case of statistical significant correlations, p-values are bold.

* The number of cases reported is affected by incidental missing cases.

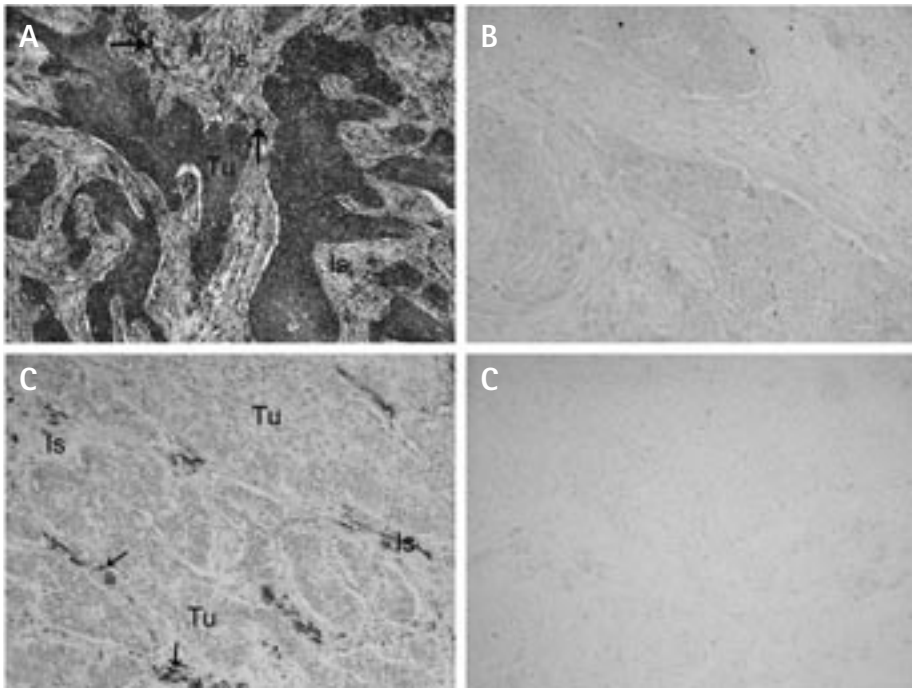

Figure 2 - TGF-β_1 mRNA in cervical carcinomas and differences in amount of intratumoral stroma. mRNA was detected by RNA in situ hybridization with an anti-sense riboprobe for TGF-β_1 as described in Material and Methods. TGF-β_1 is visualized by a blue color. (Original magnification: x 100).

A. Strong expression of TGF-β_1 mRNA in the cytoplasm of the tumor cells (Tu) is associated with a considerable amount of intratumoral stroma (Is). Note inflammatory cells within the stroma also expressing TGF-β_1 mRNA (arrow).
B. Negative control, the same tumor hybridized with a TGF-β_1 sense riboprobe.
C. Weak expression of TGF-b1 mRNA in the cytoplasm of the tumor cells (Tu) is associated with only a small amount of intratumoral stroma (Is). Note inflammatory cells within the stroma showing strong TGF-b1 mRNA expression (arrow).
D. Negative control, the same tumor hybridized with a TGF-b1 sense riboprobe.

The tumors consisted of tumor cell nests surrounded by widely varying amounts of stroma, which ranged from 6 to 82 % with a mean of 43% (Figs 1 and 2). All tumors showed a tumor infiltrate, of which 15 cases showed an extensive, 34 cases a moderate and 50 cases a minor tumor infiltrate (Table 2).

Of the examined ECM proteins in the tumor stroma fibronectin was most strongly expressed, as 49 cases showed > 75% immunoreactivity, 28 cases showed 25-75% immunoreactivity and 20 cases showed < 25% immunoreactivity. Collagen IV was less strongly expressed with 92 cases demonstrating <25% immunoreactivity, 6 cases showing 25-75% immunoreactivity and one case showing > 75% immunoreactivity. Laminin was weakest expressed with 96 cases showing < 25% immunoreactivity and 3 cases showing 25-75% immunoreactivity (Table 2).

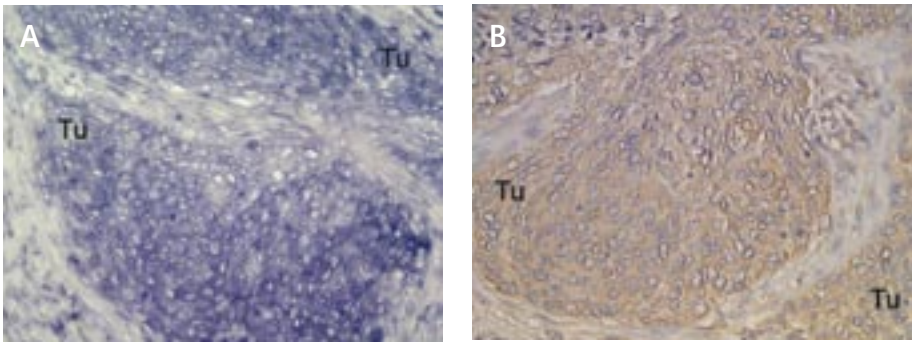

Figure 3 – TGF-β_1 mRNA expression in cervical tumor cells and corresponding PAI-1 protein expression in the same tumor. mRNA was detected by RNA in situ hybridization with an anti-sense riboprobe for TGF-β_1 and PAI-1 protein was detected by immunohistochemistry as described in Material and Methods. (Original magnification: x 200).

A. Expression of TGF-β_1 mRNA in the cytoplasm of cervical tumor cells (Tu). TGF-β_1 is visualized by a blue color.
B. Expression of PAI-1 protein in the cytoplasm of cervical tumor cells (Tu). PAI-1 expression is visualized by a brown color.

In addition, the expression of *TGFR-I* and *-II* in tumor cells was investigated by immunohistochemistry. In general, much more cytoplasmatic staining than membraneous staining was seen. Strong staining for both receptors' protein could be detected in the cytoplasm of almost all tumor cells in all tumors with little distinction in staining intensity. Since practically no difference could be observed among the tumors we did not include the scoring results into the statistical analysis.

Relationship between TGF-β_1 and histological parameters

TGF-β_1 expression by tumor cells had a statistically significant linear relationship with the total percentage of stroma within the tumor ($p \leq 0.005$) (Fig 1). Table 2 shows the results of the correlation of strong TGF-β_1 staining pattern with expression of ECM proteins. Strong TGF-β_1 expression (a total score of 7 or 8) was associated with deposition of collagen IV in the intratumoral stroma (p 0.026), but not with deposition of fibronectin or laminin. There was a positive correlation between TGF-β_1 mRNA expression and PAI-1 protein staining in the tumor cells, as all TGF-β_1 positive tumors showed PAI-1 expression (Fig 3). No TGF-β_1 positive/ PAI-1 negative tumors or TGF-β_1 negative/PAI-1 positive tumors were observed. However, semiquantitatively the amount of TGF-β_1 mRNA and PAI-1 expression in tumor cells did not demonstrate a significant relationship, nor was there a significant relationship between the amount of TGF-β_1 mRNA expression in tumor cells and PAI-1 expression in tumor stroma. Strong TGF-β_1 expression was significantly (inverse) correlated with the extent of the tumor infiltrate (p –0.04; Table 2).

Discussion

Since a striking difference in the amount of intratumoral stroma and also a marked difference in extent of the tumor infiltrate among cervical carcinomas is observed, we studied whether these phenomena could be explained by the paracrine effect of TGF-β_1 produced by cervical carcinoma cells. Our results showed a strong positive relationship between TGF-β_1 production by the tumor cells and the amount of intratumoral stroma. The stroma of carcinomas differs from that of comparable normal organs and is assumed to be an important factor in malignant growth. Although all cancers contain some stroma, certain cancers are characterized by their propensity to form dense masses of stroma around and between the invading malignant growths.[45] To date it is still not clear whether the presence of an extensive stromal reaction is only advantageous for the tumor (nourishment and gasexchange), or also represents a defense mechanism by the host.[46] Indeed Tang *et al*. have shown that expression of thymidine phosphorylase (TP), a promoter for microvessel growth, is significantly related to micro vessel density (MVD) in the tumor stroma of cervical cancer. High MVD was positively related to lymph node metastasis and poorer prognosis in that study.[47] It is thought that carcinoma cells can have an instructive influence on cells in the tumor stroma and vice versa.[9,45] Furthermore, by limiting the influx of inflammatory cells, the tumor stroma may provide a barrier to immunologic rejection, which, in case of the immunogenic type of tumor that cervical carcinoma represents, can play an important role.

The results of ECM expression in the tumor stroma that we detected were in agreement with those of Goldberg *et al.*[7] This group previously described in their study of 71 squamous carcinomas that 100% of the tumors showed peritumoral staining for fibronectin protein, while only 17% demonstrated peritumoral laminin and collagen IV expression. We also found fibronectin to be most prominently expressed in all tumors, whereas collagen IV and laminin were only expressed in part of the tumors and also to a weaker extent. The expression of fibronectin and laminin was not caused by a direct effect of tumor cell derived TGF-β_1, since no significant relationship could be detected between the expression of those two glycoproteins and the production of TGF-β_1 in tumor cells. Probably these molecules were produced by peritumoral stromal cells as has been reported by others [48,49] and perhaps (partly) stimulated to do this by tumor cell derived TGF-β_1.[9,45] There was, however, a significant correlation between tumor cell expressed TGF-β_1 and a more prominent deposition of collagen IV in the tumor stroma. This formation of a desmoplastic intratumoral stroma, desmoplasia being defined as a (extensive), collagenous and cellrich tumor stroma,[45] caused by TGF-β_1, is observed in different other types of cancer too. Löhr *et al*. showed that TGF-β_1 de-

rived from pancreatic cancer cells induced desmoplasia in pancreatic carcinoma.[46] The connection between desmoplasia in mammary cancer and the production of TGF-β_1 and TGF-β_1- induced connective tissue growth factor (CTGF), was also detected by Frazier *et al*., who demonstrated that stromal rich tumors were positive for both factors, while tumors negative for expression of both factors lacked significant stroma.[50] The fact that collagen deposition in the tumor stroma can be caused by the paracrine effect of TGF-β_1 production of carcinoma cells has also been subscribed by Hagedorn *et al*.[51] Their investigations demonstrated collagen immunoreactivity in the tumor stroma to be significantly correlated with TGF-β_1 production in tumor cells, but not in stromal cells. This paracrine effect of TGF-$\beta_{1,}$ by which tumor cells force the surrounding stroma cells to organize the tumor stroma, is also described in mammary carcinoma cell lines.[52,53] Since it is well known that TGF-β_1 is chemotactic for fibroblasts,[11,45] cervical cancer cell derived TGF-β_1 probably attracts fibroblasts to the tumor stroma and activates these cells to produce collagen IV. This mechanism could explain both the increase in stroma as well as the desmoplastic change of the tumor stroma. It is possible that such a desmoplastic change in the tumor stroma, due to the extra fibrous consistency, might be useful for more effectively walling off the tumor cells from the immunologic host defense. However, since only a small percentage of the tumors in our group demonstrated this specific fibrous stroma, this mechanism might merely be of significance in a subgroup of cervical tumors.

We have demonstrated an inverse relationship between TGF-β_1 production by tumor cells and the extent of the tumor infiltrate. de Visser *et al*., stated that TGF-β_1 dose-dependently inhibits the generation of cytotoxic T lymphocytes (CTLs) and proliferation of T lymphocytes.[15] TGF-β_1 is also known to suppress the normal function of macrophages as antigen presenting cells (APCs).[15] In colon cancer it is thought that macrophages distributed along the invasive margin are functioning as antigen-presenting cells to stimulate T cells.[54] Since T lymphocytes and macrophages make up for the major part of the inflammatory infiltrate in carcinomas of the uterine cervix,[55] the inhibitory effect of tumor cell derived TGF-β_1 on generation and proliferation CTLs and T-cells might partly explain the more limited tumor infiltrates observed in TGF-β_1-rich tumors. Furthermore, if the same mechanism holds true for cervical cancer, paracrine TGF-β_1 diminishing the function of macrophages as APCs to stimulate T cells might further restrict the tumor infiltrate. However, to better understand these phenomena, the effects of paracrine TGF-β_1 on the different components of the tumor infiltrate in cervical cancer need to be researched more extensively in future.

In conclusion, we have demonstrated that cervical carcinoma cells, via the paracrine effect of TGF-β_1, are capable of augmenting the intratumoral stroma and decreasing the tumor infiltrate. These biological phenomena might be beneficial for tumor growth and metastasis and suggest an additional mechanism for tumor cells to escape from the host's immune system.

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Chapter 4