The Potential Role of TGFβ1, TGFβ2 and TGFβ3 Protein Expression in Colorectal Carcinomas

Correlation with Classic Histopathologic Factors and Patient Survival

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Purpose: This study investigates the expression of tumor growth factors TGFβ1, TGFβ2 and TGFβ3 in tissue material from patients with colorectal carcinoma and evaluates their correlation with known prognostic markers and patient survival.
Patients and Methods: The study included 124 patients with colorectal carcinoma. According to the TNM classification of malignant tumors, 26 tumors were identified as being stage I, 30 stage II, 48 stage III, and 20 stage IV, whereas 106 tumors were low-grade and 18 high-grade malignancies. On paraffin sections, the streptavidin-biotin technique using antibodies against TGFβ1, TGFβ2 and TGFβ3 was applied. Morphological and immunohistochemical results were correlated with clinicopathologic parameters.
Results: TGFβ1 protein was expressed in 88 out of 124 (71%) carcinomas, whereas TGFβ2 and TGFβ3 proteins were detected in all tumors examined. Normal colonic mucosal epithelial cells expressed TGFβ2 (significantly less as compared to neoplastic cells; $p < 0.01$) and TGFβ3 ($p > 0.05$ compared to neoplastic cells), but not TGFβ1. Statistical analysis revealed a higher expression of TGFβ1 in low-grade carcinomas ($p = 0.009$) and a higher presence of TGFβ2 in advanced tumors ($p = 0.008$). TGFβ1 expression was related with increased disease-free and overall survival ($p < 0.05$ each). The presence of TGFβ2 was correlated with worse prognosis ($p < 0.05$). Cox analysis revealed that besides tumor grade and stage, TGFβ1 expression constituted an independent prognostic factor.
Conclusion: This study shows that in adenocarcinomas of the colon, there is a differential expression of TGFβ1, TGFβ2 and TGFβ3. TGFβ1 may be implicated in the pathogenesis of these tumors, since it is expressed only in neoplastic but not in normal cells. TGFβ1 is related with an increased disease-free and overall survival and constitutes an independent prognostic factor. In advanced stages, TGFβ2 seems to be involved in tumor progression and is related with worse prognosis.

Key Words: Colorectal carcinoma · Transforming growth factor beta · Prognostic factors

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Potentieller Stellenwert der TGFβ1-, TGFβ2- und TGFβ3-Expression bei Kolorektalkarzinomen. Korrelation mit klassischen histopathologischen Faktoren und Überleben

Ziel: Diese Studie untersuchte die Expression der Tumorwachstumsfaktoren TGFβ1, TGFβ2 and TGFβ3 in Gewebeproben von Patienten mit kolorektalen Karzinomen und prüfte ihre Korrelation mit bekannten prognostischen Markern und mit dem Überleben der Patienten.
Patienten und Methodik: Die Studie umfasste 124 Patienten mit kolorektalen Karzinomen. Nach der TNM-Klassifikation wurden 26 Tumoren als Stadium I, 30 als Stadium II, 48 als Stadium III und 20 als Stadium IV eingeordnet, während 106 Tumoren Low-Grade- und 18 High-Grade-Malignome waren. Paraffinschnittpräparate wurden nach der Streptavidin-Biotin-Methode mit Antikörpern gegen TGFβ1, TGFβ2 und TGFβ3 behandelt. Die morphologischen und immunhistochemischen Befunde wurden mit klinisch-pathologischen Parametern korreliert.

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Ergebnisse: TGFβ1 wurde in 88 von 124 (71%) Karzinomen exprimiert, während TGFβ2 und TGFβ3 in allen untersuchten Tumoren gefunden wurden. Normale Epithelzellen der Dickdarmschleimhaut exprimierten TGFβ2 (signifikant weniger verglichen mit neoplastischen Zellen; $p < 0,01$) und TGFβ3 ($p > 0,05$ verglichen mit neoplastischen Zellen), aber kein TGFβ1. Die statistische Analyse ergab stärkere TGFβ1-Expression in Low-Grade-Karzinomen ($p = 0,009$) und eine verstärkte Präsenz von TGFβ2 in fortgeschrittenen Tumoren ($p = 0,008$). Die TGFβ1-Expression korrelierte mit verlängertem krankheitsfreien und Gesamtüberleben (jeweils $p < 0,05$). Das Vorliegen von TGFβ2 korrelierte mit schlechterer Prognose ($p < 0,05$). Die Cox-Analyse ergab, dass neben Tumorgrad und -stadium die TGFβ1-Expression einen unabhängigen prognostischen Faktor darstellte.
Schlussfolgerung: Diese Studie zeigt für Adenokarzinome des Kolons und Rektums Unterschiede in der Expression von TGFβ1, TGFβ2 und TGFβ3. TGFβ1 könnte in der Pathogenese dieser Tumoren eine Rolle spielen, da es nur in neoplastischen, nicht aber in normalen Zellen exprimiert wird. TGFβ1 geht mit verlängertem krankheitsfreien und Gesamtüberleben einher und ist ein unabhängiger prognostischer Faktor. In fortgeschrittenen Stadien scheint TGFβ2 für die Tumorprogression relevant zu sein und ist mit einer schlechteren Prognose verbunden.

Schlüsselwörter: Kolorektalkarzinom · Transforming Growth Factor beta · Prognostische Faktoren

Introduction

Colorectal carcinoma is one of the most common cancers equally affecting men and women, and its prognosis is related to several clinical and pathologic parameters. Traditionally, the tumor stage, the histological type and the grade of differentiation were the main parameters for predicting prognosis as well as planning optimal therapeutic approaches. In addition, other variables such as primary tumor size, tumor margins, degree of peritumoral lymphocytic infiltration, angioinvasive growth, number and location of lymph node metastases, DNA ploidy, oncogene expression, and cell proliferation have also been used to pursue prognostic information [21, 22]. The standard therapy for colorectal carcinoma is surgical resection, the type of surgery depending on the tumor site [22]. Besides surgery, however, adjuvant treatment (consisting of radio- and chemotherapy) is also instituted; more specifically, in cases of rectal carcinoma adjuvant treatment may be given pre- or postoperatively [3, 11, 25].

During malignant tumor progression, abnormal growth factor and cytokine expression occurs. Tumor-produced cytokines include transforming growth factor beta (TGFβ). TGFβ is a member of a large family of related factors that affect several functions at the cellular level both in neonatal and adult organisms [5, 15]. In humans, the TGFβ family includes three factors with similar structures and functions: TGFβ1, TGFβ2 and TGFβ3. In vitro, these factors induce cell cycle arrest in normal and some malignant epithelial cells by inducing inhibitors of cyclin-dependent kinases [5, 9, 10, 20, 23, 32]. In addition, it is important that malignant epithelial cells including those of gastrointestinal origin display acquired resistance to the growth-inhibitory effects of TGFβ [5, 15]. The latter is accomplished by either inactivating mutations or the downregulation of TGFβ receptors, or by deletion or mutation of elements of the TGFβ signal transduction pathways [1, 2, 5, 12, 15–18, 31, 35]. Previous studies have shown that these three factors exhibit different and non-overlapping actions during embryonic development [24]. In humans, many malignant tumors overexpress TGFβ1 [4, 8, 14]. Previously, we have shown that in cases of colorectal carcinoma, TGFβ1 is produced actively and specifically by neoplastic cells, whereas normal colonic mucosal epithelial cells do not express TGFβ1 protein or mRNA [33].

This study investigates the immunohistochemical expression of TGFβ1, TGFβ2 and TGFβ3 proteins in tissue specimens of colon adenocarcinomas and their possible relation with classic histopathologic factors and patient survival.

Patients and Methods

In this study, 124 consecutive surgical specimens of primary colorectal carcinomas were included and examined, from an equal number of patients who underwent surgical excision at the University Hospital of Patras, Greece, during the period between 1990–1998. Archival tissues and data derived from the pathology records as well as clinical follow-up were readily available for all patients. There were 79 men and 45 women aged 25–82 years (median age 66 years). None of the patients received any neoadjuvant chemotherapy or preoperative radiotherapy. 51 tumors were located in the rectum, 21 in the sigmoid, 37 in the right colon, and 15 in the left colon. The slides and the pathology report for each patient were drawn out from the files of the Pathology Department and reviewed in order to confirm the pathologic grade and stage. Two pathologists (ACT, PR) did the review in a blind fashion. According to the TNM classification of malignant tumors [7], 26 tumors were identified as being stage I (14 tumors T1 N0 M0, and twelve tumors T2 N0 M0), 30 stage II (T3 N0 M0), 48 stage III (18 tumors T1–2 N1–2 M0 and 30 tumors T3 N1–2 M0), and 20 stage IV (any T any N M1). According to a modification in the grading system proposed by the WHO [13], 106 adenocarcinomas were low-grade (well and moderately differentiated) and

18 high-grade malignancies (poorly differentiated). Patients were followed for a period ranging from 3 to 148 months (median 68 months).

All 51 patients with rectal carcinoma underwent postoperative radiotherapy using a 6-MV linear accelerator and an isocentric three- or four-field technique, in prone position with the bladder distended. Radiotherapy was initiated 2–3 weeks after the first six weekly fractions of chemotherapy. The dose was 45 Gy to the tumor bed, perirectal tissues and regional lymph nodes (1.8 Gy per fraction, five fractions per week). An additional dose of 5.4 Gy was given as a boost to the tumor bed. The total dose was prescribed at the 95% or 90% isodose curve encompassing the target volume. Chemotherapy (5-fluorouracil 450–500 mg/m^2, leucovorin 200 mg/m^2) was given postoperatively up to a total dose of 24 weekly fractions, unless the patient was voluntarily withdrawn or unacceptable toxicity occurred. Additionally, 5-fluorouracil (400 mg/m^2) was administered as a single rapid infusion on the first 3 and the last 3 days of radiotherapy as a radiosensitizer.

Detection of TGFβ1, TGFβ2 and TGFβ3 protein expression relied on immunohistochemistry performed on 4 mm thick paraffin sections from one selected block per case; this block contained neoplastic and nonneoplastic colonic tissue. In tumors stage III and IV, stains were also performed in blocks from lymph nodes with metastatic disease in order to record any possible differences in the expression of all three forms of TGFβ in primary and metastatic foci. Sections were dewaxed in xylene, hydrated through graded concentrated alcohol, and quenched with H_2O_2 (0.6%) in 100% methanol for 20 min to inhibit endogenous peroxidase activity. For antigen retrieval, the slide sections were processed in a microwave oven twice for 5 min each time at high power. Subsequently, the sections were washed three times with phosphate-buffered saline (PBS), saturated in 0.1% bovine serum albumin (PBS/BSA; Sigma, Dorset, UK) for 30 min, and incubated for 30 min with the primary TGFβ1, TGFβ2 and TGFβ3 antibodies (all dilutions 1 : 100, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). Thereafter, sections were incubated with the biotinylated multilink anti-IgG immunoglobulin (diluted 1 : 80, Biogenex, San Ramon, CA, USA) and streptavidin-peroxidase complex (diluted 1 : 80, Biogenex) for 30 min each. All incubations were performed at room temperature. Between the single steps, sections were washed in PBS. 3,3′-diaminobenzidine tetrachloride (Sigma Fast DAB tablets, D-4293, St. Louis, CA, USA) was used as the chromogen. For negative control purposes, the same streptavidin-biotin technique was used in tissue sections where 1% BSA in PBS replaced the primary antibody. Cytoplasmic staining for TGFβ1, TGFβ2 and TGFβ3 was considered positive.

Cases were regarded as positive if at least 5% of tumor cells displayed cytoplasmic staining for TGFβ1, TGFβ2 and TGFβ3. All immunohistochemical slides were analyzed using a method that has been described previously [26]. Briefly, tissue sections were scanned with a light microscope at low power and areas with positive staining were selected. Cell counts were performed at a 400× magnification using a 10 × 10 microscope grid. Both the number of positive immunostained cells and the total number of cells (at least 500 cells) at selected areas were determined by visual inspection of five different fields per section. For each field, a percent value for TGFβ1, TGFβ2 and TGFβ3 in neoplastic and nonneoplastic colonic tissue was obtained by dividing the positive cells by the total number of cells counted. The values in the same field did not differ by > 10%. The average scores were then calculated. All sections were screened by two pathologists (ACT and PR) independently and scored in a blind fashion without knowledge

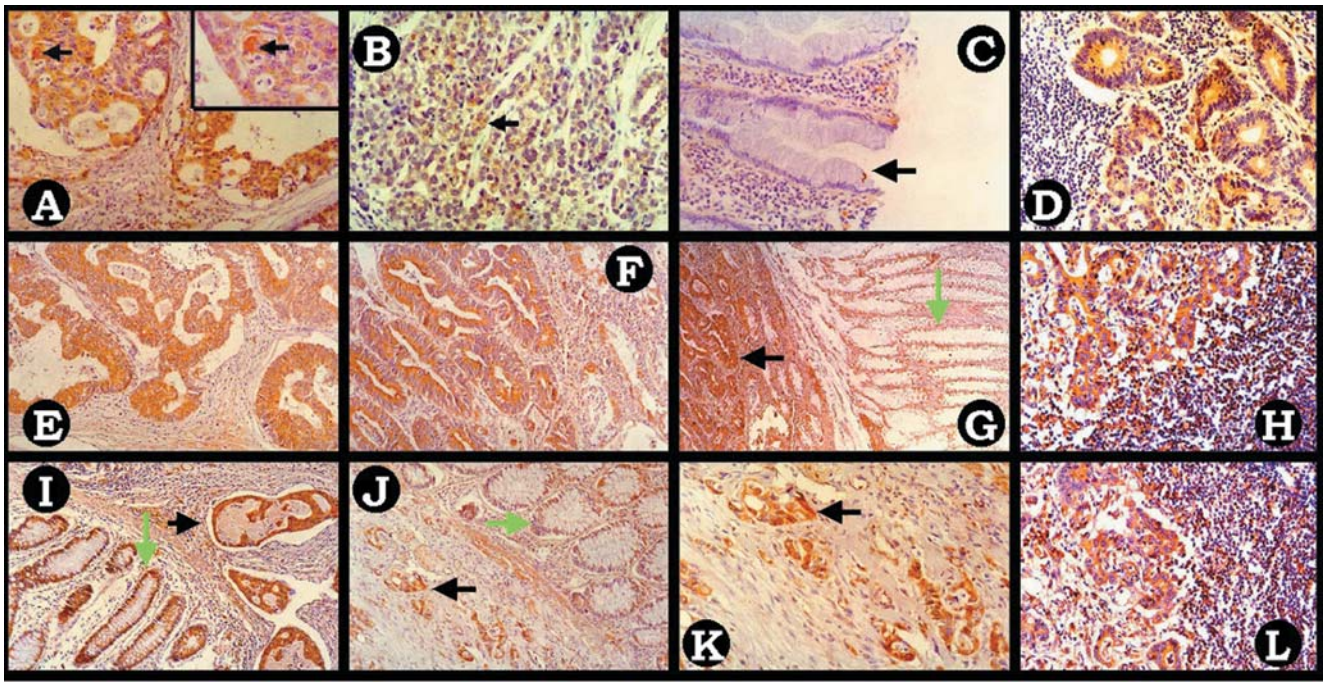
Table 1. TGFβ1, TGFβ2 and TGFβ3 protein expression in relation to various pathologic parameters. LC: left colon; R: rectum; RC: right colon; S: sigmoid.

Tabelle 1. TGFβ1-, TGFβ2- und TGFβ3-Expression in Relation zu verschiedenen pathologischen Parametern. LC: linkes Kolon; R: Rektum; RC: rechtes Kolon; S: Sigmoid.

Condition	Number of cases	TGFβ1 [number of (+) cases (mean ± SD)]	TGFβ2 [number of (+) cases (mean ± SD)]	TGFβ 3 [number of (+) cases (mean ± SD)]
Normal colonic mucosa	124	0 (0)	124 (15.6 ± 2.5)*	124 (43.3 ± 4.1)
Tumor location				
R	51	36 (38.3 ± 8.5)	51 (36.3 ± 9.7)	51 (40.7 ± 12.1)
S	21	15 (37.3 ± 9.5)	21 (37.8 ± 8.6)	21 (41.2 ± 12.3)
RC	37	26 (35.5 ± 10.3)	37 (33.3 ± 9.2)	37 (44.1 ± 10.7)
LC	15	11 (39.6 ± 11.2)	15 (39.5 ± 11.3)	15 (43.6 ± 9.5)
Tumor stage				
I				
• T1 N0 M0	14	9 (32.5 ± 7.1)	14 (23.4 ± 5.1)[b]	14 (41.3 ± 11.5)
• T2 N0 M0	12	8 (38.7 ± 3.8)	12 (27.4 ± 3.9)	12 (42.3 ± 13.1)
II				
• T3 N0 M0	30	21 (38.9 ± 2.6)	30 (34.7 ± 6.1)	30 (42.3 ± 13.1)
III				
• T1–2 N1–2 M0	18	13 (36.3 ± 4.3)	18 (35.3 ± 4.8)	18 (40.3 ± 11.2)
• T3 N1–2 M0	30	22 (37.8 ± 6.3)	30 (37.4 ± 6.1)	30 (41.2 ± 10.7)
IV				
• Any T any N M1	20	15 (41.3 ± 8.2)	20 (44.0 ± 6.7)[b]	20 (43.2 ± 9.3)
Tumor grade				
Low (I + II)	106	76 (41.7 ± 11.4)[a]	106 (44.3 ± 10.1)	106 (40.4 ± 12.1)
High (III)	18	12 (21.1 ± 0.08)[a]	18 (41.2 ± 9.2)	18 (42.2 ± 8.3)
Total	**124**	**88**	**124**	**124**

p = 0.009 in cases with matching letter [a], and p = 0.008 in cases with matching letter [b]

*TGFβ2 expression in normal colonic mucosa was lower compared to that of tumor cells regardless of tumor stage and tumor grade (p < 0.01)



Figures 1A to 1L. A) Photomicrograph showing strong cytoplasmic TGFβ1 protein expression in a case of low-grade (moderately differentiated) colon adenocarcinoma (arrows, streptavidin-biotin peroxidase ×200, inset ×400). B) Photomicrograph showing weak cytoplasmic TGFβ1 protein expression in a case of high-grade (poorly differentiated) colon adenocarcinoma (arrow, streptavidin-biotin peroxidase ×200). C) Photomicrograph of normal colonic mucosa showing no TGFβ1 expression (arrow, streptavidin-biotin peroxidase ×400). D) Photomicrograph showing strong cytoplasmic TGFβ1 protein expression in a metastatic focus in a lymph node (streptavidin-biotin peroxidase ×200). E, F) Photomicrographs showing strong cytoplasmic TGFβ2 expression in two cases of low-grade (well differentiated) colon adenocarcinomas (streptavidin-biotin peroxidase ×200). G) Photomicrograph showing strong cytoplasmic expression of TGFβ2 within neoplastic cells (black arrow) and weak cytoplasmic expression of the same protein in the normal colonic mucosal epithelial cells (green arrow) in a case of low-grade (moderately differentiated) colon adenocarcinoma (streptavidin-biotin peroxidase ×100). H) Photomicrograph showing strong cytoplasmic TGFβ2 protein expression in a metastatic focus in a lymph node (streptavidin-biotin peroxidase ×200). I) Photomicrograph showing strong cytoplasmic expression of TGFβ3 protein within neoplastic cells (black arrow) and strong cytoplasmic expression of the same protein in the normal colonic mucosal epithelial cells (green arrow) in a case of low-grade (moderately differentiated) colon adenocarcinoma (streptavidin-biotin peroxidase ×200). J) Photomicrograph showing cytoplasmic expression of TGFβ3 protein within neoplastic cells (black arrow) and normal colonic mucosal epithelial cells (green arrow) in a case of high-grade (poorly differentiated) colon adenocarcinoma (streptavidin-biotin peroxidase ×100). K) Photomicrograph showing strong cytoplasmic expression of TGFβ3 protein within neoplastic cells (black arrow) in a case of high-grade (poorly differentiated) colon adenocarcinoma (streptavidin-biotin peroxidase ×200). L) Photomicrograph showing strong cytoplasmic TGFβ3 protein expression in a metastatic focus in a lymph node (streptavidin-biotin peroxidase ×200).

Abbildungen 1A bis 1L. A) Stark ausgeprägte zytoplasmatische TGFβ1-Expression eines mäßig differenzierten Adenokarzinoms des Kolons (Pfeile, Streptavidin-Biotin-Peroxidase ×200, Ausschnitt ×400). B) Schwach ausgeprägte zytoplasmatische TGFβ1-Expression eines schlecht differenzierten Adenokarzinoms des Kolons (Pfeil, Streptavidin-Biotin-Peroxidase ×200). C) Normale Dickdarmschleimhaut ohne TGFβ1-Expression (Pfeil, Streptavidin-Biotin-Peroxidase ×400). D) Stark ausgeprägte zytoplasmatische TGFβ1-Expression in einer Lymphknotenmetastase (Streptavidin-Biotin-Peroxidase ×200). E, F) Stark ausgeprägte zytoplasmatische TGFβ2-Expression von mäßig differenzierten Adenokarzinomen des Kolons (Streptavidin-Biotin-Peroxidase ×200). G) Stark ausgeprägte zytoplasmatische TGFβ2-Expression in neoplastischen Zellen (schwarzer Pfeil) und schwach ausgeprägte zytoplasmatische Expression des gleichen Proteins in normalen Epithelzellen der Dickdarmschleimhaut (grüner Pfeil) bei einem mäßig differenzierten Adenokarzinom des Kolons (Streptavidin-Biotin-Peroxidase ×100). H) Stark ausgeprägte zytoplasmatische TGFβ2-Expression in einer Lymphknotenmetastase (Streptavidin-Biotin-Peroxidase ×200). I) Stark ausgeprägte zytoplasmatische TGFβ3-Expression in neoplastischen Zellen (schwarzer Pfeil) und stark ausgeprägte zytoplasmatische Expression des gleichen Proteins in normalen Epithelzellen der Dickdarmschleimhaut (grüner Pfeil) bei einem mäßig differenzierten Adenokarzinom des Kolons (Streptavidin-Biotin-Peroxidase ×200). J) Zytoplasmatische TGFβ3-Expression in neoplastischen Zellen (schwarzer Pfeil) und stark ausgeprägte zytoplasmatische Expression des gleichen Proteins in normalen Epithelzellen der Dickdarmschleimhaut (grüner Pfeil) bei einem schwach differenzierten Adenokarzinom des Kolons (Streptavidin-Biotin-Peroxidase ×100). K) Stark ausgeprägte zytoplasmatische TGFβ3-Expression in neoplastischen Zellen (schwarzer Pfeil) eines schwach differenzierten Adenokarzinoms des Kolons (Streptavidin-Biotin-Peroxidase ×200). L) Stark ausgeprägte zytoplasmatische TGFβ3-Expression in einer Lymphknotenmetastase (Streptavidin-Biotin-Peroxidase ×200).

of the clinicopathologic data. When major discrepancies were recorded, a consensus score was reached by simultaneous reevaluation over a conference microscope.

Results were expressed as mean ± SD. Intergroup comparisons, regarding correlation of pathologic parameters with staining results, were performed using one-way analysis of variants (ANOVA). Whenever the equal variance test or normality tests failed, the Kruskal-Wallis nonparametric test was applied. In order to address the problem of multiple comparisons, the ANOVA and Kruskal-Wallis tests were followed by a post hoc Bonferroni test. The Spearman rank correlation was used to detect any potential relations between a) TGFβ1, TGFβ2 and TGFβ3 and b) between TGFβ2 expression and survival in months. The Kaplan-Meier procedure was also used to compare the survival curves. The latter included the survival rates and also the disease-free rates. Data were analyzed using the SPSS statistical package (SPSS©, Release 10.0.1, Chicago, IL, USA). Any p-value < 0.05 was considered significant.

Results

Immunohistochemical Expression of TGFβ1, TGFβ2 and TGFβ3 in Primary Tumors

The results are shown in Table 1. TGFβ1 was detected in the cytoplasm of neoplastic cells (Figures 1A and 1B). It was present in 88 tumors. Normal mucosal cells did not display any positivity for the TGFβ1 protein (Figure 1C). TGFβ2 protein was detected in the cytoplasm of neoplastic (Figures 1E and 1F) and normal epithelial cells (Figure 1G). TGFβ2 protein expression in neoplastic cells was higher compared to normal epithelial cells regardless of tumor location, stage and grade (Table 1; $p < 0.01$). TGFβ3 protein was detected in both normal mucosa (Figures 1I and 1J) and neoplastic tissue (Figures 1I to 1K). No statistically significant difference was recorded between TGFβ3 expression in normal mucosal and tumor cells (Table 1; $p > 0.05$). TGFβ2 and TGFβ3 were present in all tumors examined. Immunohistochemical expression of TGFβ1, TGFβ2 and TGFβ3 proteins was detected in metastatic foci of lymph nodes in all cases (Figures 1D, 1H, and 1L).

Statistical analysis revealed that TGFβ2 expression was higher toward advanced tumor stage (Table 1; $p = 0.008$). However, this was not demonstrated for the other two factors TGFβ1 and TGFβ3. TGFβ1 protein expression was higher in low-grade compared to high-grade tumors (Table 1; $p = 0.009$). No relation between the presence of TGFβ2 and TGFβ3 and tumor grade nor between tumor location and expression of all three factors was recorded. Spearman rank correlation revealed that there was only a direct correlation between TGFβ2 and TGFβ3 expression in stage III tumors (Figure 2; $r = 0.610$; $p < 0.01$).

Correlation between TGFβ1, TGFβ2 and TGFβ3 Expression and Clinical Outcome

In a follow-up period of 3–148 months after the initial surgery, 75.8% of the patients (94/124) are alive. A statistically significant association was observed between advanced stage (II vs. III: $p < 0.05$; and I vs. II vs. III vs. IV: $p < 0.01$) and grade (high vs. low: $p < 0.01$).

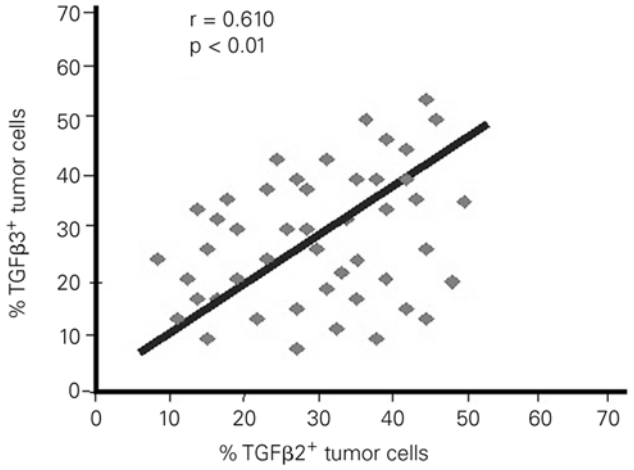


Figure 2. Spearman rank correlation showing the direct correlation between TGFβ2 and TGFβ3 expression in stage III tumors ($r = 0.610$; $p < 0.01$).

Abbildung 2. Spearman-Rank-Korrelation mit guter Korrelation zwischen TGFβ2- und TGFβ3-Expression bei Stadium-III-Tumoren ($r = 0{,}610$; $p < 0{,}01$).

Statistical correlation between the immunohistochemical results for TGFβ1 and survival showed that TGFβ1 expression was related both with longer disease-free survival and overall survival (Figures 3a and 3b; $p < 0.05$ in each case). To the contrary, TGFβ2 expression was correlated with worse survival ($r = -0.189$; $p = 0.035$; Figure 4). No correlation was found between TGFβ3 expression and patient survival or disease-free survival time.

Cox analysis of the relationship between TGFβ1, TGFβ2 and TGFβ3 values and the various clinicopathologic parameters with survival revealed that besides tumor stage and grade, of the three TGFβ isoforms only TGFβ1 constituted an independent prognostic factor (Table 2).

Discussion

This study demonstrates that in cases of colon adenocarcinoma, first, TGFβ1 may be involved in tumor pathogenesis since it is expressed specifically within neoplastic cells, second, in advanced tumor stages TGFβ2 seems to be implicated in tumor progression, and third, the expression of TGFβ1 is correlated with better survival and prolonged disease-free survival. We have also found that TGFβ1 constitutes an independent prognostic factor, both TGFβ2 and TGFβ3 proteins are expressed in neoplastic and normal colonic mucosal epithelial cells, and TGFβ2 protein expression is related with poor prognosis.

Previous studies have demonstrated that TGFβ mRNA or protein is overexpressed in colon carcinoma cells compared

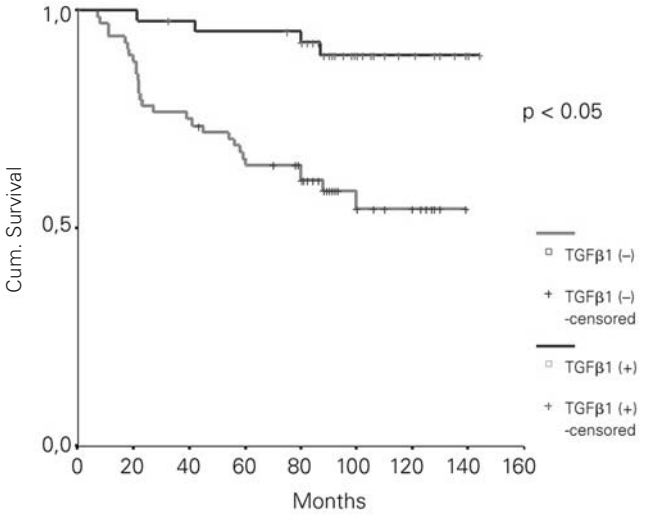


Figure 3a – Abbildung 3a

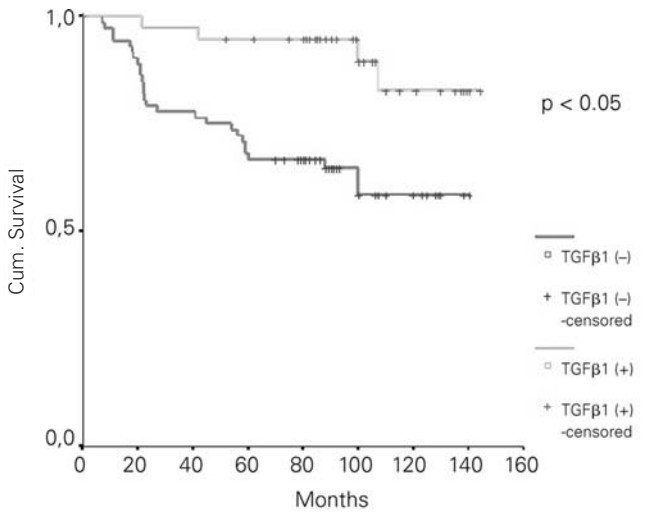


Figure 3b – Abbildung 3b

Figures 3a and 3b. Kaplan-Meier survival curves showing the relation of TGFβ1 protein presence or absence with disease-free survival (a) and overall survival (b) ($p < 0.05$ in each case).

Abbildungen 3a und 3b. Kaplan-Meier-Überlebenskurven für Patienten mit und ohne TGFβ1-Expression. Krankheitsfreies (a) und Gesamtüberleben (b) (jeweils $p < 0,05$).

to normal colonic mucosal cells [4, 6, 8, 14, 29]. Furthermore, TGFβ1 levels have been reported to be elevated in colon carcinoma [5, 27, 30, 33] and the presence of TGFβ1 has been linked with the progress and the metastatic potential of the disease [8, 34]. In addition, a recent study showed that TGFβ1 expression was higher in colon carcinomas of advanced stage [5]. However, our results did not reveal such a correlation.

A previous study, based on an animal tumor model, showed a positive correlation between tumor size and TGFβ concentration in plasma [30]. In our study, we observed strong immunostaining for TGFβ1 protein in neoplastic cells, whereas normal colonic mucosal epithelial cells were negative. This finding suggests that the activity of this factor may originate from the neoplastic cells.

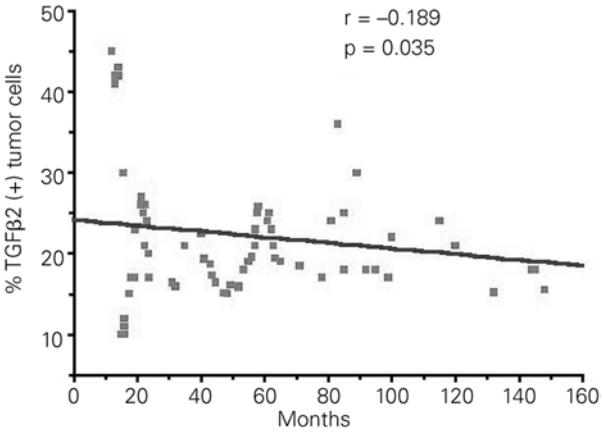


Figure 4. Spearman rank correlation showing the reverse correlation between TGFβ2 expression and survival (in months; r = —0.189; p = 0.035).

Abbildung 4. Spearman-Rank-Korrelation mit umgekehrter Relation zwischen TGFβ2-Expression und Überleben (in Monaten; r = —0,189; p = 0,035).

The results of the current study are in agreement with our previous study and suggest that TGFβ1 is involved in the pathogenesis of colon carcinoma, since it is produced actively and specifically by the neoplastic cells [33]. However, its expression was higher in low-grade compared to high-grade tumors. Thus, in high-grade colon adenocarcinomas (poorly differentiated) other mechanisms seem to be involved.

In this and in a previous study [33], TGFβ1 expression was related with increased survival and disease-free survival. In addition, Cox analysis revealed that TGFβ1 constituted an independent prognostic factor. To the best of our knowledge, these studies are the first describing such results.

By contrast, a previous study showed that TGFβ1 expression was correlated with disease progression [5]. However, these authors studied 39 patients with colorectal carcinoma and a maximum follow-up of 3 years, whereas in the current study, we included 124 patients with colorectal cancer and a maximum follow-up of 12.3 years (148 months). Thus, one can speculate that the different results regarding TGFβ1 expression and survival or tumor progression recorded in these two studies may be attributed to their different design.

On the other hand, the current study showed that TGFβ2 protein was detected both in neoplastic and normal colonic mucosal epithelial cells, but its expression in tumor cells was statistically higher. Furthermore, TGFβ2 expression was

Table 2. Relationship of potential prognostic factors with survival (Cox proportional hazards regression analysis model).

Tabelle 2. Korrelation potentieller prognostischer Faktoren mit dem Überleben (Cox-Analyse).

Factor (variable)	Coefficient	Standard error	Hazard ratio	95% confidence intervals	p
Tumor location	0.006	0.018	1.01	0.97–1.04	0.7
Tumor stage	0.591	0.276	1.81	1.04–3.03	0.02*
Tumor grade	1.56	0.63	4.9	1.42–16.0	0.02*
TGFβ1	0.425	0.405	1.55	1.33–3.08	0.025*
TGFβ2	–0.16	0.432	0.83	0.38–1.96	0.7
TGFβ3	0.248	0.413	1.28	0.56–2.91	0.5

*$p < 0.05$

higher toward advanced tumor stage. These results are somehow in agreement with a previous study [5] and suggest that TGFβ2 is involved in late stages of colon adenocarcinoma progression.

The fact that the three TGFβ isoforms exhibit different and non-overlapping actions during embryonic development [24], combined with the differences in TGFβ1 and TGFβ2 expression in normal and neoplastic colonic mucosa and their relation with prognosis, as recorded in the current study, allows us to speculate that these two growth factors (members of the same family) seem to be involved in different stages of colorectal carcinogenesis and tumor progress.

TGFβ3 presence was different from that of TGFβ1 and TGFβ2. TGFβ3 was expressed in neoplastic and normal colonic mucosal epithelial cells and the difference was not statistically significant. Furthermore, no relation was recorded between TGFβ3 presence and tumor stage or grade. Thus, it seems that TGFβ3 is not involved in tumor progression. Previous studies in knockout mice have demonstrated that targeted disruption of the mouse TGFβ1 gene results in multifocal inflammatory disease, indicating a prominent role of TGFβ1 in suppressing excessive inflammation [5, 28]. On the other hand, TGFβ3–/– mice display a mild phenotype characterized by a localized defect in epithelial cell differentiation which manifests itself in failure of the palatal shelves to fuse leading to cleft palate [5, 19]. The most severe phenotype is caused by ablation of TGFβ2 expression that results in multiple developmental defects [5, 24]. These findings suggest that the three factors (TGFβ1, TGFβ2 and TGFβ3) play different and non-overlapping roles during normal development. Subsequently, these factors may have different roles during tumor development. This may explain the differences, first, in the presence of these three factors in neoplastic and normal colonic mucosal epithelial cells and, second, in the correlation of TGFβ1 and TGFβ2 with patient survival. Thus, we are tempted to speculate that TGFβ1 is involved in the pathogenesis of less aggressive tumors (low-grade tumors), whereas TGFβ2 seems to affect tumor progression in late stages. Another significant finding is that TGFβ1 expression constitutes an independent prognostic factor. This finding, combined with the loss of TGFβ1 expression toward high-grade adenocarcinomas, implies that selective targeting of augmented TGFβ1 in low-grade (well and moderately differentiated) colon adenocarcinomas may serve as a potentially effective adjunct treatment or chemoprevention strategy.

Conclusion

This study shows that there is a differential expression in the three isoforms of TGFβ in colorectal carcinomas and this difference reflects on the malignant phenotype and, subsequently, on the survival of patients. Further studies are warranted in order to establish a clear relation between the presence of these factors and the progress of colorectal tumors.

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