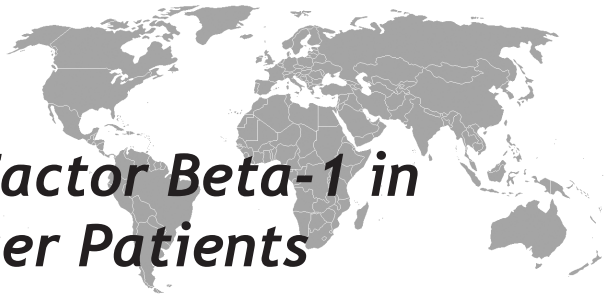

Transforming Growth Factor Beta-1 in Human Colorectal Cancer Patients

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ABSTRACT

Aim: *Colorectal cancer patients are treated with surgery and sometimes radiotherapy and chemotherapy. Transforming growth factor beta-1 (TGF-B1) acts both as an inhibitor of tumor growth and as a promoter of tumor progression. The aim of this study was to determine the levels of TGF-B1 in plasma in colorectal cancer patients and relate these to the effect of clinicopathological variables.*

Method: *One hundred patients scheduled for colorectal cancer surgery were included. Blood samples were taken during surgery and later assayed with enzyme linked immunsorbent assay for total TGF-B1 and active TGF-B1.*

Result: *Total and active TGF-B1 was higher in tumor samples compared to controls (p<0.001).Total TGF-B1 was higher in patients with metastases compared to patients without. Active TGF-B1 levels were not found statistically different in patients with metastases..*

Conclusion: *Higher levels of total TGF-B1 in plasma at surgery may be indicate of distant metastases, Measurement of total TGF-B1 in colorectal cancer patients may be of clinical use in the future.*

Key words: *Colorectal cancer, TGF-beta-1, distant metastases.*

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Kolorektal Kanserli Hastalarda Transforming Büyüme Faktörü

Amaç: *Kolorektal kanserli hastalar, cerrahi, radyoterapi ve kemoterapi ile tedavi edilirler. Transforming büyüme faktörü beta-1 (TGF-ß1), hem tümörün büyümesini inhibe eder, hem de tümör progresyonunu sağlar. Bu çalışmanın amacı, kolorektal kanserli hastalarda, TGF-ß1 düzeylerini ölçmek ve bu hastaların klinikopatolojik değişiklikler ile ilişkisi olduğunu vurgulamaktır.*
Metod: *Cerrahi uygulanan 100 kolorektal kanserli hasta incelemeye alındı. Hastaların kan örnekleri, cerrahi müdahale sırasında alındı ve sonrasında total TGF-ß1 ve aktif TGF-ß1 düzeyleri için enzim bağlı immunosorbent ölçüm (ELISA) yapıldı.*
Bulgular: *Total ve aktif TGF-ß1 düzeyleri, kolorektal kanserli hastalarda kontrollere göre yüksek bulundu (p<0.001). Total TGF-ß1 düzeyleri metastazı olan hastalarda, metastazı olmayan hastalara göre, yüksek bulundu. Aktif TGF-ß1 düzeylerinde ise, metastazı olan hastalarda istatistiksel olarak anlamlılık saptanmadı.*
Sonuç: *Plazma total TGF-ß1 ' in yüksek düzeyleri, uzak metastazın göstergesi olabilir. Kolorektal kanserli hastalarda total TGF-ß1' in ölçümü, gelecekte klinik uygulamalarda kullanılabilir.*

Anahtar kelimeler: *Kolorectal kanser, TGF-beta-1, uzak metastaz*

INTRODUCTION

Transforming growth factor-ß1 (TGF-ß1) is a multi-functional cytokine that has an important complex role in cancer cell growth and development (1,2). Two other isoforms (ß2, ß3) with sequence homology and similar functions have also been described in mammalian tissues but are much rarer (3). TGF-ß1 is secreted by most mammalian cells in a latent non-active complex from which a 25 kDa bioactive dimer can be released. TGF-ß1 appears to be a potent growth inhibitor for most cells, including epithelial, endothelial, and lymphatic cells (4). Consequently, disruption of the TGF-ß1 growth inhibitory autocrine/paracrine loop should crucially favour uncontrolled cell proliferation and transformation. This hypothesis is at present mainly supported by the frequent finding of defective alterations in the TGF-ß1 system in cancers of the stomach and colon (5), prostate (6), breast (7), and lung (8).

TGF-ß1 is produced in a latent form, where TGF-ß1 is bound to a latency-associated peptide. Activation of TGF-ß1 can be activated by endogenous agents such as plasmin or matrix metalloproteinase-9 but also exogenous through irradiation (9). After activation, TGF-ß1 is capable of performing many biological functions, such as regulation of cell growth, suppression of the immune system and remodeling of the extracellular matrix. As a regulator of extracellular matrix, TGF-ß1 is thought to contribute to the excess development of fibrosis, which is an important factor in the pathology after radiotherapy (10). TGF-ß1 has been shown to suppress tumor formation in colorectal cancer cell lines (11). However, TGF-ß1 only seems to be a tumor suppressor in the early stages of the cancer; with increasing tumor load, TGF-ß1 promotes tumor progression. It has been shown that TGF-ß1 in specimens of colorectal cancer is associated with a poor prognosis and an increased risk of recurrence and tumor progression (12, 13). The levels of both total and active TGF-ß1 have also been found to be higher in patients with colorectal cancer, and they have also been associated with disease progression (14). However, another study suggests that TGF-ß1 is only produced by colorectal tumor cells and is an independent positive prognostic factor (15). Thus, the importance of TGF-ß1 in colorectal cancer in the clinical setting remains unclear. The aim of this study was to examine the levels of total and active TGF-ß1 in plasma in patients with colorectal cancer.

MATERIALS AND METHODS

The local ethics committee approved the study, and all patients gave informed consent. One hundred patients scheduled for colorectal cancer surgery were included. All patients admitted to our unit with suspected colorectal adenocarcinoma and an indication for bowel resection was included. Exclusion criteria were distant metastases or histology other than adenocarcinoma. Medical history, clinical examination, full blood count, and a biochemical screen of renal and liver function were performed before surgery. Staging was done with chest radiography or chest spiral computed tomography (CT) and an abdominal ultrasound for colon cancer or abdominal spiral CT and endoscopic ultrasound for rectal tumors. Blood samples were taken during surgery. After centrifugation, the samples stored at -80° C until analysis. Analysis was carried out in batches for total TGF-ß1, with commercially available kits (Promega, Madison, WI). Measurements of active and total amounts of TGF-ß1 were performed in separate steps. The active

Table 1. *Patient and controls data.*

Variables	Controls	Patients	p value
Age (y)	56.2±11.4	57.9±10.1	>0.05
Male	26	54	
Female	24	46	

fraction of TGF-β1 was assayed directly in the enzyme linked immunosorbent assay (ELISA) plate using the kits provided. For measuring the total amount of TGF-β1, additional samples were acidified to pH 3.0 using 1 mol/L HCL, followed by 15 min incubation at 22° C, resulting in activation of all TGF-β1. The number of patients in each analysis is shown in each graph. Tests for statistical differences were carried out with the Mann-Whitney U analysis, Chi-squared test, Fisher's exact test and Kruskal-Wallis test. Logistic regression was used for multivariate analysis. P values below 0.5 were considered significant.

RESULTS

We found that the levels of total TGF-β1and active TGF-β1 were higher in plasma than in controls ($p<0.001$) (Table 1 and 2). Total TGF-β1 was higher in the patients with distant metastases ($p<0.001$) (Table 3). But, active TGF-β1 levels were not found significant different as statistically. No relationship was found between the other parameters and TGF-β1 levels: T stage, N stage.

DISCUSSION

This study shows that total TGF-β1 is a higher in plasma samples. High levels of total TGF-β1 in the cancer patients may indicate invasive potential. This is illustrated by the fact that we found higher levels of total TGF-β1 in the tumors of patients presenting with metastases. Our explanation is that these high levels of total TGF-β1 give the tumor an opportunity to exert a localized suppression of the immune system and thus facilitate tumor spread. Our findings are somewhat similar to those of Friedman et al and Angenete et al (12, 13), who showed a correlation between TGF-β1 and disease progression. In resected patients, TGF-β1 could work as a tumor suppressor. It has been suggested that high levels of TGF-β1 in the distal part of the colon (16) may explain the higher number of distal colon cancers compared to proximal colon cancers.

One hypothesis was that TGF-β1 in plasma would be correlated to the tumor stage and that it would be possible to use it as a predictive marker. Several studies have previously addressed the issue of plasma TGF-β1 and correlation with disease stage and progression (14, 17, 18). We found a somewhat different result in our study where univariate analysis and logistic regression showed no differences levels of active TGF-β1 in plasma in patients later developing metastases. It is possible that a lack of active TGF-β1 in plasma facilitates tumor invasion in peripheral tissue, as TGF-β1 at this stage of the disease most probably exerts its tumor-protective properties. However, our results do not deliver a cutoff value, and there is also a considerable variability as well as a small sub-group of patients. With this in mind, our results are interesting but require further study.

In conclusion, this study was found higher levels of total TGF-β1 in tumor samples. It also showed that total TGF-β1 seems to be of importance to the tumor for dissemination. Perhaps, total TGF-β1 can be used to assess tumor aggressiveness pre-operatively. Finally, the correlation between active and total TGF-β1 in plasma and development of metastases may be use in the clinical setting in the future.

Table 2. *Total and active TGF-β1 levels.*

	Controls	Patients	p value
Total TGF-β, pg/ml	3.4 ± 1.1	50.9 ± 12.1	<0.001
Active TGF-β1, pg/ml	2.5 ± 0.9	34.7 ± 16.4	<0.001

***Table 3**. Clinicopathological variables and total and active TGF-B1 levels.*

Variables	*n*	*total TGF-B1*	*active TGF-B1*
pT stage			
T1	*12*	*36.8±10.3*	*19.7±9.8*
T2	*23*	*40.6±9.9*	*23.3±10.4*
T3	*30*	*43.1±17.9*	*28.6±13.7*
T4	*35*	*48.7±18.2*	*35.8±11.1*
M stage			
M0	*49*	*31.7±12.9*	*29.5±10.3*
M1	*51*	*59.3±8.1*	*38.6±7.5*

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