



Original Contribution

PROGNOSTIC SIGNIFICANCE OF TUMOR-ASSOCIATED CHANGES IN LIVER SINUSOIDS OF PATIENTS WITH GASTROINTESTINAL CANCERS

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ABSTRACT

Liver is the main target organ for developing metastases from gastric and colorectal cancers. Homing of the disseminated tumor cells and formation of the metastatic deposits includes tumor-induced reactions of the target organs. The aim of the current study is to elucidate tumor-related changes in livers of patients with gastrointestinal cancers and evaluate their prognostic significance. Routine histology and immunohistochemistry for collagen IV, α -smooth muscle actin (α -SMA) and laminin were performed in 22 liver biopsies from patients with colorectal and gastric carcinoma. Among the histological characteristics only the increased number of lymphocytes in liver sinusoids ($p=0.021$) correlated with a shorter survival. A tendency for worse prognosis was registered in the patients with low number of Ito cells ($p=0.119$), low occurrence of α -SMA ($p=0.110$) and collagen type IV ($p=0.087$), as well as with high occurrence of laminin ($p=0.047$). All of these characteristics correlated with a low degree of differentiation, which in turn was associated with a shorter survival ($p=0.047$). In conclusion, we suggest that the observed liver changes in our study may be used as prognostic markers for the progression of gastrointestinal cancers before hepatic metastases are detected or proved.

Key words: Extracellular matrix proteins, Gastrointestinal cancers, Immunohistochemistry, Ito cells, Prognosis

INTRODUCTION

Cancer metastasis is a complex multi-step process influenced by cancer cell-related biological factors. Homing of the disseminated tumor cells and formation of the metastatic deposits includes tumor-induced reactions in the target organs long before the metastases are established and proved (1).

Liver is the main target organ for developing metastases from gastric and colorectal cancers (2). This might be explained by the filter function of the liver for circulating tumor cells (3). According to the report of Koch *et al.*, (3) the hepatic metastatic lesions emerge from disseminated tumor cells that spread from the primary colorectal tumor and reach the capillary vessels in the liver via the portal venous drainage. Here they are quantitatively

retained by filter effect; and after establishment of metastasis in the liver, disseminated tumor cells may spread further to reach the lungs and other distant organs.

Cancer cells, which are homed in the new tissue, realize very specific interactions with the molecules on the surfaces of the endothelial cells of liver sinusoids, leading to changes in the activity and protein expression of these and other host liver cells (1). Thus, Miyagawa *et al.*, have reported that the liver macrophages are accumulated and activated in peritumoral regions in patients with colorectal liver metastasis and such an accumulation and activation of liver macrophages is related to patients' poor prognosis (4). Earlier we detected several changes in sinusoids of livers of patients with gastrointestinal cancers. We observed dilated sinusoids filled with mononuclear cells, perisinusoidal fibrosis, an increase in the Ito cell population and enhanced deposits of collagen type III and IV, laminin and α -smooth muscle actin (α -SMA) in the periportal and pericentral zone of livers in patients with cancer (5, 6).

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The aim of the current study is to evaluate the significance of these tumor-related liver changes for the progression of the disease and prognosis of patients with gastrointestinal cancers.

MATERIALS AND METHODS

Patients and tissue specimens

The patient group consisted of 16 males and 6 females, who underwent surgery for adenocarcinoma of the stomach (n=10), colon (n=7) and rectum (n=5) at the Department of Surgery, Medical Faculty, Trakia University. At the time of surgery the patients were aged between 49 and 77 years. Five of the patients had stage I (TNM staging), 3 had stage II, 9 - stage III and 5 of the patients had stage IV primary tumors. Fourteen of the patients were with one or more regional lymph nodes involved in the disease, whereas only 5 of them also had distant metastases, mainly in the liver (n=3). Twelve of the patients had primary tumors with a high differentiation grade and the remaining 10 with a low differentiation grade. The median survival after the operation was 23 months ranging between 5 and 93 months. At the end of the follow-up period, 15 patients had survived.

In addition to cancer patients, 3 patients (1 man and 2 women), between 53 and 72 years old, were studied as controls. They underwent laparotomy for liver calcification, hepatic haemangioma and abdominal trauma, respectively. Informed consent was obtained from each patient.

We obtained wedge-like liver surgical biopsies, which were excised far away from metastases, if such existed (> 5 cm distance). The surgical biopsies of approximately 16x15x8 mm were taken from the lower border of the liver. Each of the biopsies was sliced in pieces, which were processed for routine histology and immunohistochemistry.

Immunohistochemistry

The light microscopical immunohistochemistry was performed as it was described earlier (5, 6). In brief: cryostat sections (5 µm thick), pretreated with normal goat serum for 60 min were incubated with the primary antibodies for 24 h at room temperature. Afterwards, they were reacted with biotinylated anti-mouse (or goat antimouse) antibodies for 4 h, and then with peroxidase conjugated streptavidin (or mouse peroxidase-antiperoxidase complexes), for 4 h at room temperature. Peroxidase activity was developed by using a freshly prepared

solution of 3-amino-9-ethyl-carbazole (AEC). As negative controls, sections incubated with non-immune sera instead of the primary antibodies were used.

Immunochemicals

The antibodies used were the following ones: mouse anti-human alpha-smooth muscle actin (AM128-5M) (BioGenex Lab, USA); mouse anti-human collagen IV (MA079-5C) (BioGenex Lab, USA); and mouse anti-human laminin (M0638) (DAKO, Denmark). The detection systems used were the immunostaining kits StrAviGen (AD000-5M) (BioGenex Lab, USA) and PAP-mouse kit (HP000-5M) (BioGenex Lab, USA). The chromogen was 3-amino-9-ethyl-carbazole (AEC) (BioGenex Lab, USA).

Histological and immunohistochemical assessment

Intensity of staining was evaluated semiquantitatively, scored in 4 grades from - to +++ (- = no staining; + = weak staining; ++ = moderate staining; and +++ = strong staining) and then, according to the level of expression, the samples were divided into two groups: with high and low immune deposits.

Ito cells were counted in 5 consecutive fields of vision of pericentral zone in semithin sections for each patient, at magnification x 400. The mean value of the scores was evaluated to represent each patient.

Statistical analysis

The results from immunohistochemistry, histological observation and clinical data were analyzed using the StatViewTM package for Windows, v.4.53 (Abacus Concepts Inc., USA). The general descriptive statistic was applied for the evaluation of the mean, median and the standard deviation. Contingency tables were analyzed by Fisher's exact test. Cumulative survival curves were drawn by the Kaplan-Meier method and the difference between the curves was analyzed by the Mantel-Cox (Log-rank) test. Results with p<0.05 were considered to be statistically significant.

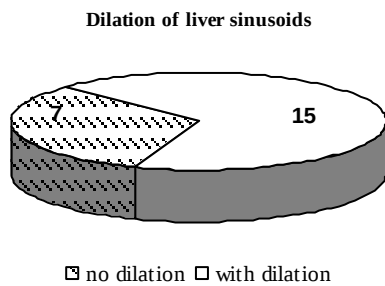
RESULTS

Histology and immunohistochemistry

In 15 of the liver biopsies dilated sinusoids were observed (Figure 1A). In all liver samples of the cancer patients, mononuclear cells were present in sinusoids (Figure 2), whereas there was no inflammatory cells in

the control samples. The inflammatory infiltration in sinusoids of cancer patients' biopsies was intense in 8 patients and with a moderate density in the remaining 14 patients (Figure 1B).

A



B

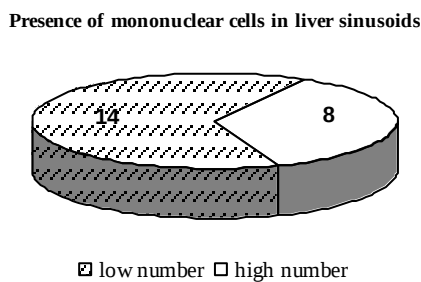


Figure 1. Distribution of routine histopathological features such dilation of sinusoids (A) and the presence of mononuclear cells in liver sinusoids (B) in liver biopsies from patients with gastrointestinal cancers.

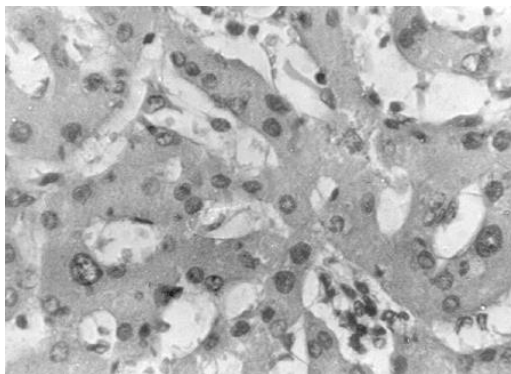


Figure 2. Mononuclear cells in sinusoids (colorectal cancer) (x 400).

Immunohistochemical investigation demonstrated increased collagen type IV deposition (Figure 3) in all patients, and in 8 of the biopsies it was greatly enhanced (Figure 4A). Laminin occurrence with different level of intensity was detected in all samples of the patients (Figure 4B). Alpha-SMA-positive Ito cells were greatly increased in number in half of the patients (higher than the median of 7.2 cells) (Figure 4C). The observed changes in the immunohisto-

chemical parameters were significantly associated with the grade of differentiation of primary tumors ($p < 0.05$, Fisher's exact test) (Table 1).

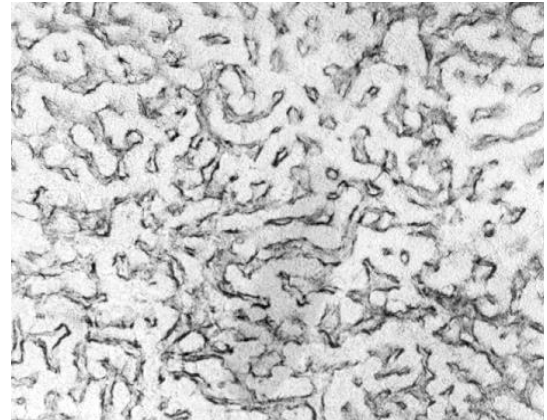


Figure 3. Collagen type IV deposits in the liver of a patient with colorectal cancer (x 100).

Survival analysis

When survival after the operation of the patients with gastrointestinal cancers was examined as a function of the observed histological changes and immunohistochemical parameters in their liver biopsies, some strong statistically significant associations were obtained. Patients with a lower number of mononuclear cells in liver sinusoids appeared to have significantly longer survival after the operation compared to those with a high number of mononuclear cells ($p = 0.021$, Logrank test) (Figure 5). Patients with low laminin deposits had a significantly better prognosis than those with high laminin expression ($p = 0.047$, Logrank test) (Figure 6A), whereas the patients with stronger collagen type IV immunoreactivity survived longer than those with weaker signal intensity ($p = 0.087$, Logrank test) (Figure 6B). A tendency for longer survival after the operation was observed in patients with a higher number of Ito cells ($p = 0.119$, Logrank test) (Figure 7A) and with higher α -SMA immunodeposits ($p = 0.110$, Logrank test) (Figure 7B) in liver biopsies than those of patients with a lower number of Ito cells and weaker α -SMA immunostaining. When the survival of the patients was analyzed in relation to the tumor cell differentiation grade, it appeared that this tumor characteristic affected the prognosis of the patients; i.e. patients with primary tumors with a high or moderate differentiation grade survived longer after the operation than the ones with a low grade of differentiation ($p = 0.047$, Logrank test) (Figure 8).

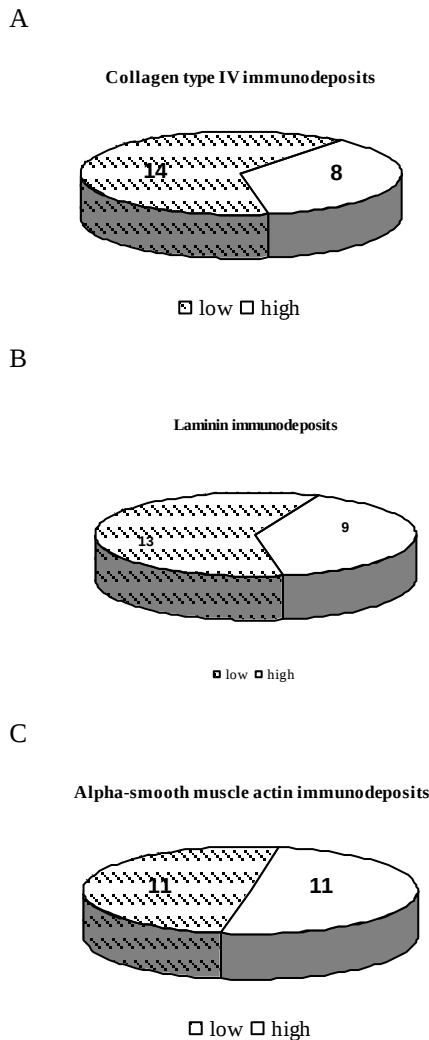


Figure 4. Distribution of the intensity of the immunohistochemical signals for collagen IV (A), laminin (B) and α -SMA (C) among the liver biopsies studied.

DISCUSSION

On the basis of histochemical and immunohistochemical investigations, we conclude that there are distinct changes in liver sinusoids of patients with gastric and colorectal cancers both with and without liver metastases. We consider that the increased number of mononuclear cells in sinusoids could be a part of the host antitumor response (7). However, the higher number of mononuclear cells observed in sinusoids can be associated with a worse prognosis of the patients after the operation than the lower number. The presence of a high number of mononuclear cells in liver sinusoids may reflect the presence of more disseminated tumor cells in the blood flow. Thus, the increased number of mononuclear cells could be used as a prognostic marker for the progression of gastrointestinal cancers.

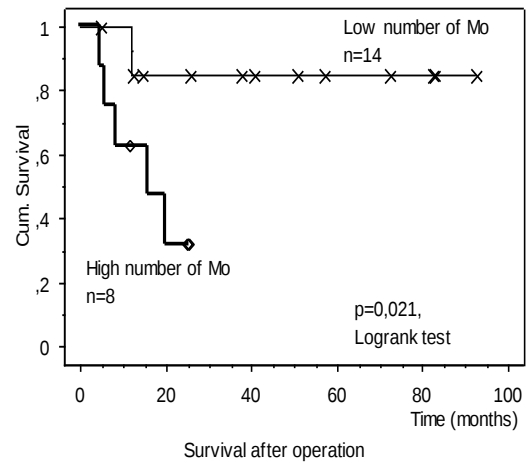


Figure 5. Survival of patients with gastrointestinal cancers as a function of the number of mononuclear cells in sinusoids of liver biopsies.

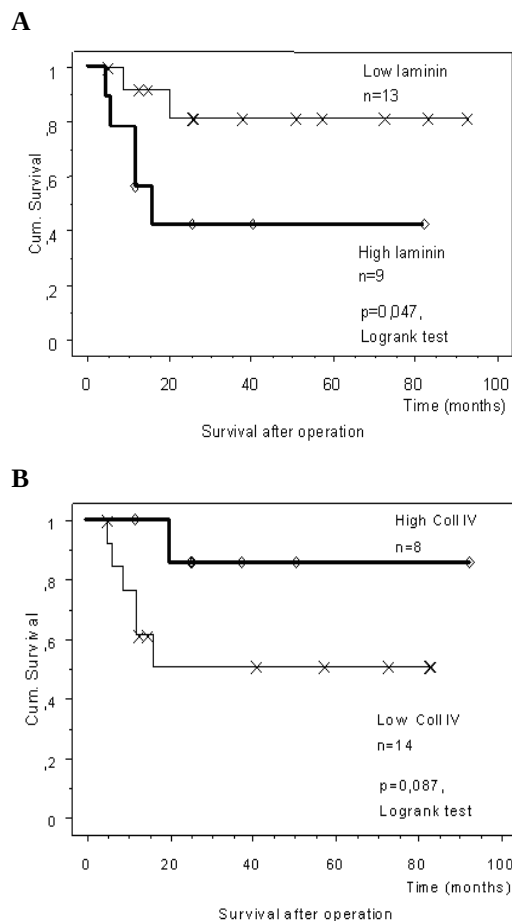


Figure 6. Association of survival of the patients with laminin (A) and collagen type IV (B) deposits in the liver biopsies.

Extracellular matrix is considered to act as a natural barrier that cancer cells must cross during invasion (1). An increased number of α -SMA-positive Ito cells was found around experimental (8) and around human hepatocellular cancer (9).

Similar observation was found in our study.

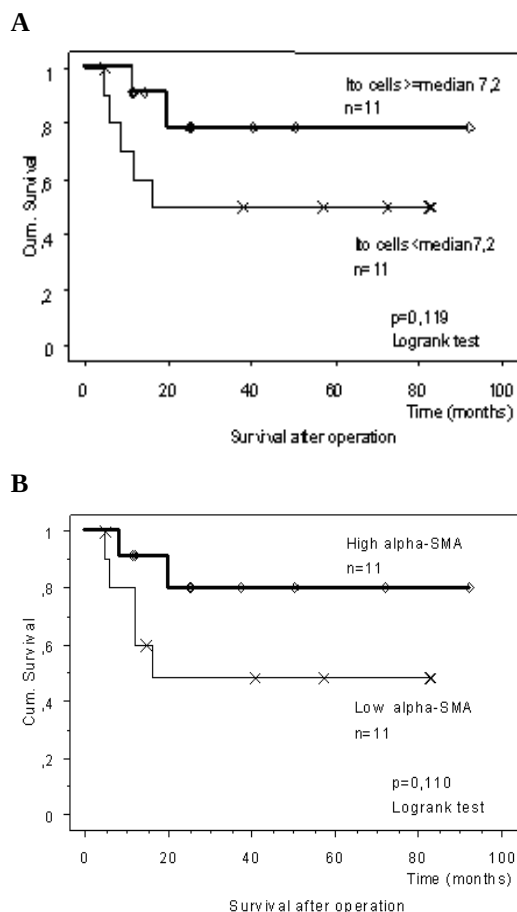


Figure 7. Survival after the operation of the patients as function of Ito cell number (A) and α -SMA immunodeposits (B).

It could be supposed that some tumor-derived stimuli activate Ito cell. A confirmation of this presumption is the enhanced accumulation of collagen type IV and the *de novo* deposition of laminin in liver sinusoids of cancer patients, found in our study. The observed correlations between the shorter survival and the lower number of Ito cells, lower deposition of α -SMA and collagen type IV as well as with the higher occurrence of laminin in liver of patients with gastrointestinal cancers, suggest the importance of these extracellular matrix proteins in inflammatory reaction and filtering function of the liver. In addition, these liver changes may be used as prognostic markers for the progression of the diseases, before hepatic metastases are detected or proved.

Since the above-mentioned liver changes are associated with a lower degree of differentiation of the primary tumors, we suppose that the disseminated tumor cells from the more aggressive low differentiated cancers inhibit the inflammatory reaction in the liver, thus contributing to the progression of the disease and shortening of the survival.

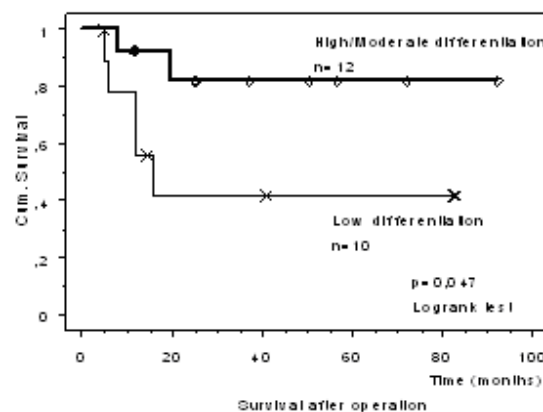


Figure 8. Correlation of the survival after operation of the patients with gastrointestinal cancers with the grade of differentiation of primary tumors.

Table 1. Association of differentiation grade of primary tumors with the immunohistochemical observations for collagen type IV, laminin and α -SMA in liver biopsies of patients with gastrointestinal cancers.

Differentiation grade	N	Collagen IV deposition		Laminin deposition		α -SMA deposition	
		Low	High	Low	High	Low	High
Low	10	10	0	3	7	10	0
High/Moderate	12	4	8	10	2	1	11
		p=0.002 Fisher's test		p=0.027 Fisher's test		p<0.0001 Fisher's test	

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