

• CLINICAL RESEARCH •

Limited influences of chemotherapy on healthy and metastatic liver parenchyma

Nancy Van Damme, Pieter Demetter, Wouter De Bock, Marianne Rottiers, Marleen Praet, Bernard de Hemptinne, Marc Peeters

Nancy Van Damme, Wouter De Bock, Marc Peeters, Department of Gastroenterology, Ghent University Hospital, De Pintelaan 185, 1K12IE, Ghent 9000, Belgium
Pieter Demetter, Marianne Rottiers, Marleen Praet, Department of Pathology, Ghent University Hospital, De Pintelaan 185, Blok A, Ghent 9000, Belgium
Bernard de Hemptinne, Department of Surgery, Ghent University Hospital, De Pintelaan 185, 2K12IC, Ghent 9000, Belgium
Correspondence to: Nancy Van Damme, PhD, Department of Gastroenterology, Ghent University Hospital, De Pintelaan 185, 1K12IE, Ghent 9000, Belgium. nancy.vandamme@ugent.be
Telephone: +32-9-2405665 Fax: +32-9-2404984
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Abstract

AIM: To examine the expression of E-cadherin, β -catenin, γ -catenin, VEGF, and p53 in 39 patients with hepatic metastasis from colorectal cancer immunohistochemically.

METHODS: The patients were divided into two groups: those ($n = 16$) who had no chemotherapy for at least 6 mo before the liver resections and those ($n = 23$) who were treated with chemotherapy before liver resections. A score from 0 to 3 was given for the number of positive cells and from 0 to 3 for the intensity of staining in these cells, in both healthy and metastatic liver parenchyma.

RESULTS: No significant differences in the expression of E-cadherin, β - and γ -catenin, VEGF and p53, could be observed between patients who received and did not receive chemotherapy, in both normal and metastatic liver parenchyma.

CONCLUSION: Despite the assumption that chemotherapy had an effect on liver metastasis, no influences were noticed immunohistochemically.

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Key words: Chemotherapy; Metastatic liver parenchyma; Cadherin; Catenin; VEGF

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INTRODUCTION

Colorectal cancer is one of the most common solid tumors and is the second leading cause of cancer deaths in Western countries. Approximately half of all patients develop metastatic disease^[1]. Lymph node or hematogenic metastasis (liver or lung metastasis) are thought to be most significant prognostic factors for patients with colorectal cancer.

Until recently, 5-fluorouracil (5-FU) and leucovorin (LV) were the standard of care, however without major impact on survival. Two new drugs, irinotecan and oxaliplatin have demonstrated survival improvement, when given either alone or in combination with 5-FU+LV, in first- or second-line therapy. Recently, Tournigand *et al.*, described that FOLFIRI (irinotecan and 5-FU/LV) followed by FOLFOX6 (oxaliplatin and 5-FU/LV) or the reverse in advanced colorectal cancer achieved a prolonged survival with a median of 24 mo and similar efficacy^[2].

The metastatic cascade of colorectal cancer starts with the infiltration of neoplastic cells through the basement membrane, which enables tumor cells to leave epithelial structures, to invade the surrounding stroma, to enter either blood or lymphatic vessels and create a metastasis in the appropriate target organs.

The cadherin-mediated cell-cell adhesion system plays a critical role in cancer invasion and metastasis^[3,4]. Cadherins are calcium-dependent transmembrane adhesion molecules that connect cells homotypically. The cytoplasmic domain of E-cadherin interacts with intracellular proteins called α -, β - and γ -catenins, which are the major elements in the intracytoplasmic microfilament network^[4,5]. Both β - and γ -catenin bind directly to E-cadherin; Alpha or-catenin links the bound β - or γ -catenin to the actin cytoskeleton, thereby forming complexes of either E-cadherin/ β -catenin/ α -catenin or E-cadherin/ γ -catenin/ α -catenin^[5]. Loss of expression of E-cadherin and catenins is found to be associated with de-differentiation, invasion, and metastasis, and is, therefore, suggested as a potential prognostic factor in colorectal carcinoma^[6,7]. β -catenin, apart from linking with E-cadherin, forms complexes with the tumor-suppressor protein - adenomatous polyposis coli (APC) gene products, which are independent from the cadherin/catenin^[8-10]. The APC gene is often mutated in colorectal cancer tissue. *In vitro* and *in vivo* data suggest a pivotal role of cadherins and catenins in various aspects of tumor progression.

Angiogenesis is required for tumor growth and metastasis of human solid tumors. This process is driven by a balance of stimulatory and inhibitory influences^[11]. The potent angiogenic factor - vascular endothelial growth factor (VEGF)

is associated with metastasis in human colon cancer^[12]. An association exists between VEGF expression, angiogenesis, and metastasis in colon cancer. Warren *et al.*, demonstrated that antibodies to VEGF inhibit metastasis in a murine model of human colon cancer^[13].

On the other hand, tumor progression is known to occur through the sequential deregulation of rearrangement of proto-oncogenes together with the inactivation of tumor suppressor genes^[14]. *In vitro* studies demonstrated the important role of p53 tumor suppressor gene in controlling tumor angiogenesis^[15,16].

Direct effect of treatment on the liver consisting of hepatocyte steatosis is a common response to liver injury and is characterized by macro- and microvesicular fatty infiltration of hepatocytes^[17]. These fatty changes may be accompanied by hepatocyte ballooning, an inflammatory infiltrate, fibrosis and even cirrhosis^[18,19].

Limited data are available about the effect of chemotherapy on healthy and malign liver parenchyma. One study described a small necrotic focus of hepatocytes on normal liver of Wistar rats who were treated with 5-FU^[20]. Therefore, in the present study we analyzed by immunohistochemistry the expression of E-cadherin, β - and γ -catenin, VEGF and p53 in normal and metastatic liver parenchyma of colorectal cancer patients. The percentage of macro- and microvesicular steatosis was also analyzed. The patients were divided into two groups: those who received chemotherapy and those who did not.

MATERIALS AND METHODS

Patients

Paraffin-embedded tumor specimens from 39 patients with liver metastasis of colorectal carcinoma who had undergone surgery were retrieved from the archival material of the Department of Pathology of the Ghent University Hospital. The characteristics of primary tumors are listed in Table 1. The mean age of patients at the time of liver metastasis diagnosis was 60 ± 12 years (range 28–81 years). The study population consisted of 27 males and 12 females. Sixteen patients received no chemotherapy for at least 6 mo before the liver resections. Twenty-three patients received chemotherapy for at least 6 mo before the liver resections (Table 2). The study was approved by the Ethical Committee of the Ghent University Hospital.

Table 1 Colorectal cancer patients' characteristics

	Number
Sex (M/F)	27/12
Location	
Sigmoid	12
Rectum	6
Rectosigmoid	4
Cecum	4
Colon ascendens	3
Colon descendens	1
Splenic feature	2
Bauhin's valve	1
Not specified	6

Table 2 Twenty-three patients received chemotherapy before the liver resection

Type of chemotherapy	Number
5-FU, LV, oxaliplatin	7
5-FU, LV, CPT-11	2
5-FU, LV	5
Cetuximab, FOLFIRI	1
Cetuximab, CPT-11	1
CPT-11	1
Oxaliplatin, capecitabine, CPT-11	1
Others	5

5-FU: 5-fluorouracil, LV: leucovorin.

Immunohistochemical staining

Monoclonal anti-E-cadherin, anti- β -catenin, and anti- γ -catenin antibodies (clones 36, 14, and 15, respectively) were obtained from BD Biosciences (San Diego, CA, USA). Monoclonal anti-VEGF antibody (C-1) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Monoclonal anti-p53 antibody was obtained from Biogenex (San Ramon, CA, USA). Immunohistochemical staining was based on the labeled streptavidin-biotin method and carried out using a DAKO LSAB[®] +Kit (DAKO Corporation, Carpinteria, CA, USA). Tissue sections (5 μ m) were deparaffinized with xylene and dehydrated in graded ethanol. For unmasking the antigens, the slides were heated in a microwave oven in citrate buffer (0.01 mol/L, pH 6) (except for anti-VEGF staining). Endogenous peroxidase activity was quenched by incubating in 0.3% hydrogen peroxidase for 20 min. Slides were then incubated with appropriately diluted antibodies for 30 min (anti- γ -antibody) and 1 h (for the other molecules). Immunostaining was visualized using diaminobenzidine tetrahydrochloride solution (for p53) or 3-amino-9-ethylcarbazole (for the other molecules); both products were obtained from DAKO. The sections were counterstained with hematoxylin. Isotype-specific irrelevant antibodies were used to control nonspecific binding of the primary antibodies.

Histology of liver parenchyma

Histology of the liver parenchyma was reviewed for the presence of macro- and microvesicular steatosis. The extent of steatosis was determined as the percentage of hepatocytes involved, in the range of 10%.

Evaluation and analysis

Sections were examined by light microscopy and the same scoring system was used for the hepatocytes and the metastatic cells. A score from 0 to 3 was given for the number of positive cells and from 0 to 3 for the intensity of staining in these cells. The sum of both scores, with a maximum of 6, was made^[21]. The differences in the numerical data between the two groups (chemotherapy or no chemotherapy) were evaluated using the Mann-Whitney *U* test. Values are expressed as median and range. *P* values of less than 0.05 were considered statistically significant.

RESULTS

Expression of the adhesion molecules in normal and metastatic liver parenchyma

Table 3 summarizes the expression of E-cadherin, β - and

γ -catenin in normal and metastatic liver parenchyma. One patient was not evaluated in the metastatic liver parenchyma due to the low number of metastatic cells. E-cadherin was expressed in 100% in normal and in 97% of the cases in metastatic liver parenchyma (Figure 1). β -catenin was expressed in 90% of the cases in normal and in 95% of the cases in metastatic liver parenchyma. γ -catenin was expressed in 46% of the cases in normal and in 66% of the cases in metastatic liver parenchyma. VEGF was expressed in 100% in normal and in 84% of the cases in the metastatic liver parenchyma (Figure 2). No staining for p53 was found in normal liver parenchyma, and p53 staining was found in 66% of the tumors (Figure 3).

Table 3 Positive staining of the adhesion molecules in normal and metastatic liver parenchyma

	Normal (%)	Metastatic (%)
E-cadherin	39/39 (100)	37/38 (97.37)
β -catenin	35/39 (89.74)	36/38 (94.74)
γ -catenin	18/39 (46.15)	25/38 (65.79)
VEGF	39/39 (100)	32/38 (84.21)
p53	0/39 (0)	25/38 (65.79)

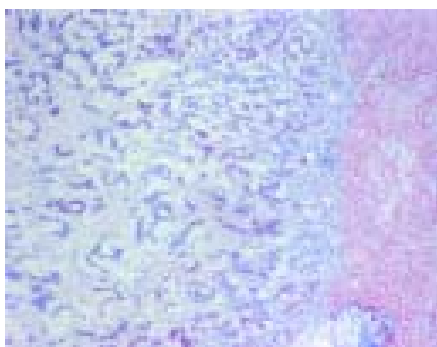


Figure 1 E-cadherin expression in a liver metastasis. Magnification x40.

Effect of chemotherapy on the expression of adhesion molecules and macro- and microvesicular steatosis

The patients were divided into two groups: those who received no chemotherapy ($n = 16$) and those who received chemotherapy ($n = 23$) for at least 6 mo before the liver resection. Both in normal and in metastatic liver parenchyma, no significant differences in the expression of adhesion molecules could be observed between the patients who received and who did not receive chemotherapy (Table 4).

Also, no significant differences were observed in macro-

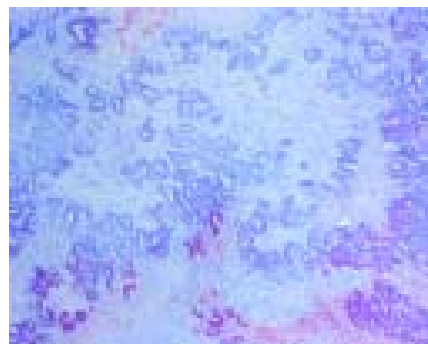


Figure 2 VEGF expression in a liver metastasis. Magnification x40.

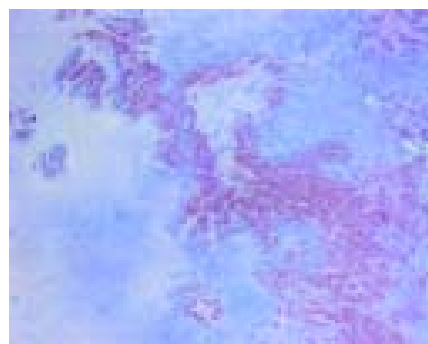


Figure 3 p53 expression in a liver metastasis. Magnification x40.

Table 5 Percentage of macro- and microvesicular steatosis (median and range)

	Chemotherapy ($n = 23$, %)	No chemotherapy ($n = 16$, %)
Macrovesicular	10 (0-40)	10 (0-30)
Microvesicular	0 (0-10)	0 (0-10)

and microvesicular steatosis between patients receiving chemotherapy and no chemotherapy (Table 5).

DISCUSSION

Invasion and metastasis of tumor cells are the primary causes of fatal outcome of cancer. E-cadherin plays a major role in the cell-cell adhesion of normal epithelial cells. The reduced expression of E-cadherin has been correlated with de-differentiation, tumor invasion, and metastasis in various cancer tissues. Several immunohistochemical studies describe a reduced expression of the E-cadherin/ α -catenin in primary

Table 4 Expression of the adhesion molecules in normal and metastatic liver parenchyma (median and range)

	Normal		Metastatic	
	Chemotherapy ($n = 23$)	No chemotherapy ($n = 16$)	Chemotherapy ($n = 23$)	No chemotherapy ($n = 16$)
E-cadherin	3 (2-4)	3 (2-6)	3 (0-6)	4 (2-6)
β -catenin	3 (0-5)	3 (0-5)	6 (0-6)	5 (0-6)
γ -catenin	0 (0-3)	1 (0-2)	2 (0-4)	2 (0-4)
VEGF	5 (3-5)	5 (3-5)	4 (0-5)	3 (0-4)
p53	0 (0-0)	0 (0-0)	3 (0-6)	2.5 (0-6)

colorectal carcinomas^[6,7,22,23]. In contrast, the marked reduction in E-cadherin and α -catenin, β - and γ -catenin were seldom reduced^[7]. Hugh *et al.*, described that α -catenin expression was reduced or lost more frequently than either E-cadherin or β -catenin on 60 colorectal cancer specimens^[24].

Little information is available about the expression of E-cadherin/catenin complex in colorectal cancer liver metastases. Ghadimi *et al.*, found a reduced E-cadherin and α -catenin expression in 48% and 36% of the cases, respectively^[7]. The expression of β - and γ -catenin was preserved in colorectal liver metastases in more than 85% and 96% of the cases, respectively. Gofuku *et al.*, described a reduced expression of E-cadherin/ α -catenin in metastatic liver tumors^[6]. In contrast, Ikeguchi *et al.*, described an increased expression of E-cadherin, α - and β -catenin in metastatic liver tumors^[23].

In the present study, we analyzed by immunohistochemistry the expression of E-cadherin, β - and γ -catenin in normal and metastatic liver parenchyma of colorectal cancer patients. The expression of E-cadherin and β -catenin was preserved in 97% and 95% of the cases in metastatic liver parenchyma, respectively. γ -catenin expression was reduced in 34% of the cases. Re-expression of E-cadherin, α -catenin and β -catenin, but not of γ -catenin was found in metastatic tissue from breast cancer patients^[25]. Mayer *et al.*, reported that E-cadherin expression in the metastatic liver tumors was stronger than in the primary tumors in many cases of gastric cancer^[26]. Another study in advanced gastric cancer showed a re-expression of the cadherin/catenin complex in lymph nodes with metastasis^[27]. The mechanism behind this re-expression remains unclear. Mareel *et al.* showed that tumor cells re-express E-cadherin at the metastatic site^[28]. Whether the same mechanism is true for catenins is not known. Re-expression of these adhesion molecules by tumor cells after release from the primary site may be important and perhaps necessary for tumor cells to adhere in remote organs.

Over the past decade, numerous studies have demonstrated that the neo-vascularity of tumors correlates with aggressiveness and metastatic potential^[29-31]. VEGF is one of the critical factors in determining the angiogenic phenotype in a majority of human colon cancers. The overexpression of VEGF is correlated with liver metastasis and poor prognosis in colorectal cancer patients^[32,33]. The most common genetic alteration in colorectal cancer is reported to be the loss of p53 tumor suppressor gene function^[14]. Protein p53 has various important functions in cellular aggregation, including cell growth control, response to DNA damage, regulation of transcription and control of genetic stability.

In the present study we analyzed by immunohistochemistry the expression of VEGF and p53 in normal and metastatic liver parenchyma of colorectal cancer patients. The expression of VEGF and p53 was seen in 84% and 66% of the cases in metastatic liver parenchyma, respectively. VEGF and p53 expression status coincided in 19 of 39 tumors (48%). Kang *et al.*, described that the incidence of liver metastasis was very high, 52% in patients whose primary colorectal tumors were both p53 and VEGF positive^[34].

No data are available in the literature about the effect of chemotherapy on the expression of adhesion molecules. In the present study, 23 patients received chemotherapy

for at least 6 mo before the liver resection. No significant differences in the expression of E-cadherin, β -, γ -catenin, VEGF and p53 could be observed between patients receiving and patients receiving no chemotherapy before the liver resection. We could not make a distinction in type of chemotherapy due to small groups of patients. Further investigation is necessary with greater number of patients in each type of chemotherapy.

Despite the assumption that chemotherapy had an effect on the liver metastasis, no significant differences were observed immunohistochemically on the expression of E-cadherin/catenin complex, VEGF and p53.

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