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Prognostic Value of E-Cadherin, Beta-Catenin, MMPs (7 and 9), and TIMPs (1 and 2) in Patients With Colorectal Carcinoma

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Background and Objectives: Therapy of colorectal tumors (CRC) based on histology and clinical factors is insufficient to predict the evolution of each patient. The finding of molecular abnormalities able to differentiate subgroups of patients with bad prognosis will improve our ability to treat them successfully. Our purpose was to analyze retrospectively the prognostic input of E-cadherin, β-catenin, metalloproteinases (MMPs) (7 and 9), and tissue inhibitors of metalloproteinases (TIMPs) (1 and 2) in patients with a follow-up period of 5 years.

Methods: Antigen expression was analyzed by immunohistochemistry. Prognostic evaluation was performed with the multivariate proportional hazards model.

Results: We demonstrated a concomitant loss of E-cadherin and β-catenin at membranous level and an abnormal accumulation of nuclear β-catenin. Besides, we found that all MMPs and TIMPs studied were overexpressed in CRC tissue. There was no association between the expression of any of these molecules and the known clinical-pathological parameters employed in CRC pathology. A multivariate analysis demonstrated that the overall survival could be independently predicted by the loss of E-cadherin and the overexpression of TIMP-2.

Conclusions: The expression of E-cadherin and TIMP-2 could be relevant in determining the prognosis of CRC patients and providing a more accurate mechanism for their classification.

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KEY WORDS: **tumor marker; colorectal cancer; E-cadherin; metalloproteinases; TIMPs**

INTRODUCTION

Colorectal carcinoma (CRC) incidence varies considerably throughout the world, being one of the leading cancers in developed countries. The age-standardized mortality rates (per 100,000) of CRC for Argentina (period 1997–2001) are 14.5 and 9.0 cases in men and women, respectively, being the third most common cause of cancer-related death [1]. Thirty to 60% of patients with CRC undergoing primary surgery with curative intention still die from metastatic disease [2]. Several clinical factors are ordinarily employed for assessing individual CRC prognosis, such as pathologic state, histologic type,

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grade, vasoinvasive status of the tumor, and status of the surgical resection margins [3]. However, these tumor parameters are insufficient to predict the evolution of each individual patient. So it is of high priority to find tumor markers able to differentiate subtypes of early CRC allowing the selection of patients with bad prognosis to apply an earlier adjuvant therapy.

It is widely known that tumor formation is a complex, multistep process involving the accumulation of genetic lesions in genes that regulate the pathways of cell proliferation, adhesion, differentiation, and death required for normal development. Glycoproteins involved in the cell–cell and cell–extracellular matrix (ECM) adhesion of tumoral cells are believed to participate in the acquisition of an invasive and metastatic phenotype. In this sense, the E-cadherin/β-catenin complex has a critical role in cell–cell adhesion. E-cadherin, a transmembrane protein that mediates the calcium-dependent adhesion of cells, is specifically expressed in epithelia and is involved in maintenance of their phenotype. β-catenin binds directly to the cytoplasmic domain of E-cadherin and to the actin microfilament network of the cellular cytoskeleton. This binding is essential for stable cell–cell adhesion [4]. Loss of E-cadherin may result in reduced cell adhesiveness and increased invasion [4].

β-catenin is a critical component of the highly conserved Wnt signaling pathway that regulates cell proliferation and differentiation [5]. A protein complex, composed by APC protein, GSK3β, and AXIN promotes degradation of free cytosolic β-catenin through phosphorylation of its NH_2 terminal sequence and its subsequent ubiquitination and proteosome degradation. When the Wnt pathway is activated, β-catenin ubiquitination and degradation are inhibited and β-catenin enters the nucleus where it complexes with transcription regulator proteins activating target genes such as cyclin D, PPAR γ, gastrin, and the proteolytic enzyme MMP-7 [6–8].

In order to invade, epithelial cancer cells need to penetrate through the basement membrane (BM) and to disorganize the ECM. In this context, proteases play a key role since they can either degrade or process the ECM components and thereby support cancer cell invasion [9]. It is well known that tumor cells produce higher amounts of proteolytic enzymes than their normal counterparts. The metalloproteinases (MMP) are enzymes deeply involved in the acquisition of the invasive phenotype. MMPs are a large group of zinc-containing endopeptidases with a central role in the degradation of all types of ECM components. Among them, the gelatinases or type IV collagenases, MMP-2 (gelatinase A, 72 kDa) and MMP-9 (gelatinase B, 92 kDa) are capable of degrading components of the BM, particularly type IV collagen, the first vital barrier breached by tumor cells when they become invasive [10]. Besides, gelatinases are a multifunctional group of biologically important molecules endowed with functions other than merely cleaving the ECM, such as the modulation of angiogenesis or tumor growth [11]. The activities of these enzymes are well regulated by endogenous tissue inhibitors of metalloproteinases (TIMPs) [12].

The aim of this study was to analyze the expression pattern of E-cadherin, β-catenin , MMPs (7 and 9), and their natural endogenous inhibitors TIMPs (1 and 2) in a group of 84 uniformly treated patients with CRC tumors, 35 of them with localized disease, with a follow-up period of 5 years for survivors. Results were correlated with clinical and anatomo-pathological parameters accepted as established prognostic factors, including survival rate.

MATERIALS AND METHODS

Patients and Tumors

Eighty-four paraffin embedded CRC tumors corresponding to 1993–1999 period were collected from the "Hospital Italiano de Buenos Aires." All tissue specimens were obtained by resection from untreated patients at initial diagnosis and were classified both morphologically and according to the stage. Patients were selected at random from a data base that included both survivors at 5 years and patients that relapsed in a shorter time. All patients, except patients with CRC stage IV, had surgery with curative intent and presented a tumor cell-free surgical resection margin. Two patients of stage II with histological factors of bad-prognosis, patients stage III and IV received adjuvant chemotherapy after surgery (5-FU 400 mg/m^2 and Leukovorin 20 mg /m^2, days 1–5, every 28 days). All patients who died had clear evidence of uncontrolled tumor growth at the time of death. Table I shows some features of the studied population.

Immunohistochemistry (IHC)

Tumor specimens were fixed in 10% formaldehyde in PBS immediately after removal and processed to paraffin blocks. Representative serial sections (5 μm thick) were placed on positively charged glass slides and microwaved in citrate buffer (pH = 6) to recover antigenicity. Sections were incubated with the following commercial primary antibodies: anti E-cadherin and β-catenin (C20820 and C19220, respectively, Transduction Research[Q1]), anti phospho β-catenin (Ser33); anti MMP-9 and anti TIMP-1 (sc-16743, sc-6840, and sc-6832, respectively from Santa Cruz[Q2]Biotechnologies); anti MMP-7 (MAB3315, Chemicon[Q3]) and anti TIMP-2 (clone 3A4, Novo Castra[Q4]). Then, sections were incubated with biotinylated anti-mouse or anti-rabbit antibody, according to the species of the first antibody (Gibco, BRL, Gaithersburg, MD). After

TABLE I. Characteristics of the Studied Colorectal Carcinoma (CRC) Population

			Sex	
Stage	n (84)	Age median (range)	M (n = 47)	F (n = 37)
I	18	55 (32–72)	9	9
II	17	70 (55–78)	10	7
III	36	66 (46–79)	19	17
IV	13	70 (60–77)	9	4

washing, sections were treated with Vectastain ABC kit Universal (PK-6200, Vector Laboratories, Burlingame[Q5]) and then incubated with the chromogen 3,3′diamino-diaminobencidine (DAB). Finally, they were counter-stained with Harris hematoxylin. Negative controls, missing out the first antibody or incubating with primary not related antibodies, were performed to discriminate background staining.

The expression of all antigens was analyzed by three independent observers and was scored according to the number of cells with positive bright brownish staining. Differences in the intensity of staining were not considered. The labeling index for each antigen was calculated as the percentage of labeled cells out of the total number of tumor cells counted. For some statistical analysis, scores were later dichotomized to a score of ''negative'' or ''positive'' staining. For E-cadherin and β-catenin a value of 10% was required before a case was accepted as positive. For MMPs (7 and 9) and TIMPs (1 and 2) the threshold of positivity was raised to 25%.

Statistical Analysis

We analyzed the relationship between the expression of the different antigens and the known prognostic factors in CRC: TNM stage (according to the American Joint Committee on Cancer, Cancer Staging Manual, Fifth Edition, 1997), differentiation grade [well differentiated (G1), moderately differentiated (G2), poorly differentiated (G3), and undifferentiated (G4)], tumor location [(ascending, sigmoid, rectum, and others (descending, transverse and coecum)], complications (perforation, bleeding, obstruction, or multiple), type of invasion (lymphatic, venous, perineural, or multiple), serosal invasion and the number of metastatic lymph nodes (0, 1–3, or >3). Taking in account that surgery was always successful and that adjuvant therapy was homogeneous for each stage (no treatment for stage I patients and adjuvant therapy for all stage III and IV patients), except for stage II where only 2/17 patients received the same systemic treatment, we considered that the variable therapy was already included in the variable stage and we did not include it in the statistical analysis.

For this analysis the Chi-square test and Pearson's correlation coefficients were employed. A difference of $P < 0.05$ was considered as significant.

The Kaplan–Meier method was used to estimate survival, defined as the time between tumor diagnosis and the patient's death or last contact at 5 years. In univariate survival analyses, two-sided log rank tests for equality of survivor functions were used to assess the prognostic significance of different parameters on antigen positivity. Multivariate analysis was performed using the stepwise Cox proportional hazards model to evaluate the predictive power of each variable independently of the others. All variables were entered in the multivariate analysis as categorical ones.

SPSSPC+ (version 10) for Windows software was used for the aforementioned analyses.

RESULTS

CRC Immunostaining of E-Cadherin and β-Catenin

The expression of E-cadherin and β-catenin was analyzed in 84 patients with colorectal carcinomas of different stages. In the normal colonic mucosa, distant at least 1 cm from the tumor, E-cadherin and β-catenin were uniformly present at the epithelial cell borders. E-cadherin specific antibody preferentially stained the plasma membrane of tumor cells. Forty-two percent of CRC samples showed a low expression of E-cadherin. Regarding β-catenin, we found that only 50% of samples were positive for its expression at membrane level. Employing a bivariate Pearson correlation test, we found that the expression of E-cadherin and β-catenin at membrane level were positively correlated ($P < 0.01$).

Interestingly, 55% of the CRC specimens showed staining for nuclear β-catenin, either with a diffuse pattern or forming speckles. Statistical analysis indicated a significant inverse correlation between the percentage of cells with membranous β-catenin immunostaining and that with nuclear expression of the antigen (Pearson, $P < 0.005$).

Additional studies showed that the phosphorylated form of β-catenin was exclusively localized in the cytoplasm (data not shown).

Possible relationships between antigens immunostaining and clinico-pathological features were analyzed, classifying cases as positive or negative according to the 10% cut-off. No significant association between E-cadherin and β-catenin labeling index at membrane level and the different prognostic factors was found. As an example, some associations are shown in Table II.

Expression of MMPs and TIMPs in CRC

A subgroup of 60 CRC cases was analyzed for the expression of MMPs and TIMPs. Adjacent ''normal''

TABLE II. Relationship Between the Expression of E-Cadherin and β-Catenin and Some Relevant Clinico-Pathological Features in the Prognosis of CRC

Parameter	n = 84	E-cadherin negative (%)[a]	β-catenin negative (%)[a]
Stage			
I	18	8 (44.4)	8 (44.4)
II	17	6 (35.3)	9 (52.9)
III	36	16 (44.4)	19 (52.8)
IV	13	5 (38.5)	7 (53.8)
Differentiation grade			
G1	14	6 (42.9)	6 (42.9)
G2	47	19 (40.4)	26 (55.3)
G3/G4	15	6 (40.0)	10 (66.7)
Metastatic lymph nodes			
0	37	15 (40.5)	19 (51.4)
1–3	28	10 (35.7)	14 (50.0)
>3	19	10 (52.6)	10 (52.6)
Localization (ascending)			
Colon	13	4 (30.8)	5 (38.5)
Sigmoid	20	11 (55.0)	12 (60.0)
Rectum	46	18 (39.1)	22 (47.8)
Other	5	3	1
Complications			
No	27	13 (48.1)	13 (48.1)
Perforation	7	3 (42.9)	5 (71.4)
Bleeding	40	16 (40.0)	21 (52.5)
Obstruction	6	2 (33.3)	2 (33.3)
Multiple	3	3	2
Serosal invasion			
Yes	55	24 (43.6)	31 (56.4)
No	27	10 (37.0)	12 (44.4)
Type of invasion			
No	50	21 (42.0)	25 (50.0)
Lymphatic	21	9 (42.9)	11 (52.4)
Venous	4	2	2
Perineural	2	1	1
Multiple	7	2 (28.6)	4 (57.1)

[a]No significant association was found (Chi-square test).

peritumoral colonic epithelium exhibited a weak expression of the gelatinase MMP-9 and the matrylisin MMP-7. On the contrary, CRC tumors presented a high number of cells stained, at cytoplasmic level, for both antigens. Thus, 49/60 (81.7%) and 32/60 (53.3%) CRC tumors were positive for MMP-7 and MMP-9 respectively, according to the chosen cut-off value.

TIMPs (1 and 2) labeling was also found in the cytoplasm of the tumor cells, showing either a highly granular or a diffuse quality. Almost all CRC cases showed some degree of staining for TIMP-1 and TIMP-2.

Interestingly staining was not restricted to epithelial tumor cells. We observed specific staining for all antigens in the extracellular matrix of the tumor stromal compartment. Moreover, we found a positive correlation between the expression of MMPs at cellular and stromal level. Similar results were observed for TIMPs expression.

Correlation analysis showed a significant positive association between MMP-9 and MMP-7 ($P < 0.05$) and between MMP-7 and nuclear β-catenin ($P < 0.05$). Besides, statistical analysis did not reveal any significant association between MMPs or TIMPs expression and the different known prognostic factors (Table III).

A subgroup of 38 CRC cases was also analyzed for the expression of MMP-2. We observed a high immunopositivity that correlated with MMP-9 and TIMP-2 expression (data not shown).

Uni and Multivariate Analysis of Survival

Survival of CRC patients has been shown to be mainly dependent on the stage of the disease at the time of diagnosis. Staging involves the following criteria: tumor depth, presence and number of lymph node metastasis, and presence or absence of distant metastasis. In the CRC studied population the 5-year survival rate was 94.7% for stage I, 77.8% for stage II, and 57.9% for stage III, while stage IV carried a 0% 5-year survival rate.

TABLE III. Relationship Between the Expression of MMPs (7 and 9) and TIMPs (1 and 2) and Some Relevant Clinico-Pathological Features in the Prognosis of CRC

Parameter	n = 60	MMP-9 positive (%)	MMP-7 positive (%)	TIMP-1 positive (%)	TIMP-2 positive (%)
Stage					
I	10	4 (40.0)	7 (70.0)	3 (30.0)	5 (50.0)
II	14	7 (50.0)	11 (78.6)	5 (35.7)	11 (78.6)
III	23	10 (43.5)	20 (87.0)	9 (39.1)	11 (47.8)
IV	13	11 (84.6)	11 (84.6)	4 (30.8)	6 (46.2)
Differentiation grade					
G1	8	4 (50.0)	7 (87.5)	2 (25.0)	5 (62.5)
G2	38	23 (60.5)	30 (79.0)	13 (34.2)	21 (55.3)
G3/G4	12	4 (33.3)	10 (83.3)	5 (41.7)	4 (33.3)
Metastatic lymph nodes					
0	26	13 (50.0)	21 (80.8)	9 (34.6)	17 (65.4)
1–3	17	9 (52.9)	14 (82.4)	5 (29.4)	11 (64.7)
>3	17	10 (58.8)	14 (82.4)	7 (41.2)	5 (29.4)
Localization (ascending)					
Colon	10	5 (50.0)	8 (80.0)	4 (40.0)	5 (50.0)
Sigmoid	16	10 (62.5)	13 (81.3)	8 (50.0)	6 (37.5)
Rectum	33	16 (48.5)	28 (84.8)	9 (27.3)	21 (63.6)
Other	1	1	0	0	1
Complications					
No	20	13 (65.0)	19 (95.0)	8 (40.0)	12 (60.0)
Perforation	6	3 (50.0)	4 (66.6)	3 (50.0)	4 (66.7)
Bleeding	27	12 (44.4)	23 (85.1)	8 (29.6)	12 (44.4)
Obstruction	4	1	2	1	3
Multiple	3	2	1	1	2
Serosal invasion					
Yes	44	26 (59.1)	38 (86.4)	17 (38.6)	22 (50.0)
No	15	6 (40.0)	11 (73.3)	4 (26.7)	10 (66.7)
Type of invasion					
No	36	16 (44.4)	27 (75.0)	11 (30.6)	19 (53.3)
Lymphatic	15	9 (60.0)	15 (100.0)	7 (46.7)	10 (66.7)
Venous	3	2	3	1	1
Perineural	1	0	0	0	0
Multiple	5	4	4	2	3

No significant association was found (Chi-square test).

We analyzed the value of the expression of the different antigens on survival. Each analysis was done independently because the known biological interactions among the different molecules could interfere with the results.

Univariate analysis demonstrated a significant correlation between E-cadherin overexpression and overall survival (OS) at 5 years (Fig. 1). On the contrary, differences in survival were not statistically significant either for membranous or nuclear β-catenin, even when each stage was analyzed separately (data not shown). When the multivariate Cox proportional hazards analysis was performed, the loss of E-cadherin demonstrated to be an independent prognostic factor associated with worse OS (Table IV).

Kaplan–Meier curves for MMPs indicated that expression of MMPs was not associated with survival (data not shown). An interesting observation was that CRC patients whose tumors expressed MMP-7 in more than 50% of the cells showed an association with worse OS but with a borderline significance (Rank test 3.19, $P = 0.07$).

On the other hand, patients survival analysis for CRC overexpressing both TIMP-1 and TIMP-2 showed a higher risk of death at 5 years (Fig. 2). Multivariate Cox analysis indicated that only TIMP-2 overexpression remained as a statistically significant prognostic factor after adjustment for the effects of the main prognostic factors in CRC pathology (Table V).

Our findings were validated because the multivariate analysis showed that high stage, high number of metastatic lymph nodes and the presence of lymphatic, venous, or perineural invasion were indicative of a shorter survival time in the studied CRC population, as other authors have found in other group of patients.

DISCUSSION

CRC is by far the most common malignancy of the gastrointestinal tract and it is without question, a "surgical disease." An estimated 92% of colon cancer patients and 84% of rectal cancer patients undergo surgical resection

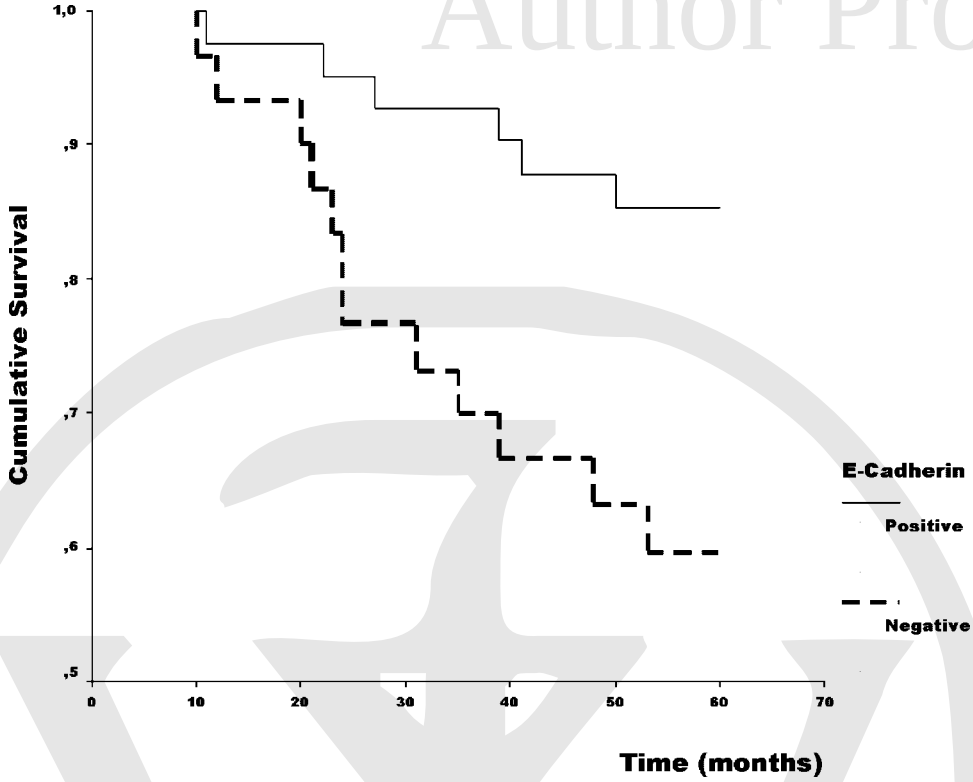

Fig. 1. Kaplan–Meier curves of overall survival categorized by E-cadherin labeling index. Tumors were classified as positive when ≥10% of the cells were specifically stained. Difference in survival was statistically significant (Log rank test 6.31, $P < 0.01$).

as the primary modality of treatment. The prediction of outcome for the patient is, to a large extent, based on the pathologic assessment of the local disease and other tissue-based prognostic factors in the resection specimen [3]. But, despite the prognostic power of these parameters, the outcome for patients with tumors of similar stage is heterogeneous. To more accurately individualized prognosis additional tissue-based prognostic indicators have been sought on a molecular level. In this sense, in the present work, we studied the expression of key molecules, associated with the invasive process and their role in determining the outcome of CRC patients. We analyzed a group of homogeneously treated patients that included a high number of individuals with low stages, with a recording of high-quality clinical data and with a 5-year follow-up for the survivors.

It is known that normal epithelial cells express the transmembrane glycoprotein E-cadherin, that mediates the calcium-dependent adhesion between cells, and its intracellular partners catenins at the cell membrane [13].

TABLE IV. Cox Survival Model for E-Cadherin Overexpression in CRC Patients

Independent variable	Coefficient	*P*	Relative risk	95% confidence interval
E-cadherin	−0.88	0.005	0.42	0.23–0.76
Sex	0.13	0.81	1.14	0.39–3.35
Stage	0.71	0.014	2.04	1.15–3.59
Differentiation grade	−0.05	0.90	0.95	0.42–2.14
Localization	0.31	0.31	1.36	0.75–2.47
Complication	−0.24	0.69	0.79	0.25–2.49
Invasion	0.48	0.035	1.61	1.05–2.48

In the present analysis, the variable number of metastatic lymph nodes was not included in the analysis because this variable is included in the stage. A new COX study replacing stage by the number of metastatic lymph nodes maintained E-cadherin as an independent tumor marker and confirmed that the presence of metastatic lymph nodes is an independent prognostic marker [Coef 1.06, $P = 0.05$, RR: 2.90 (1.38–6.07)].

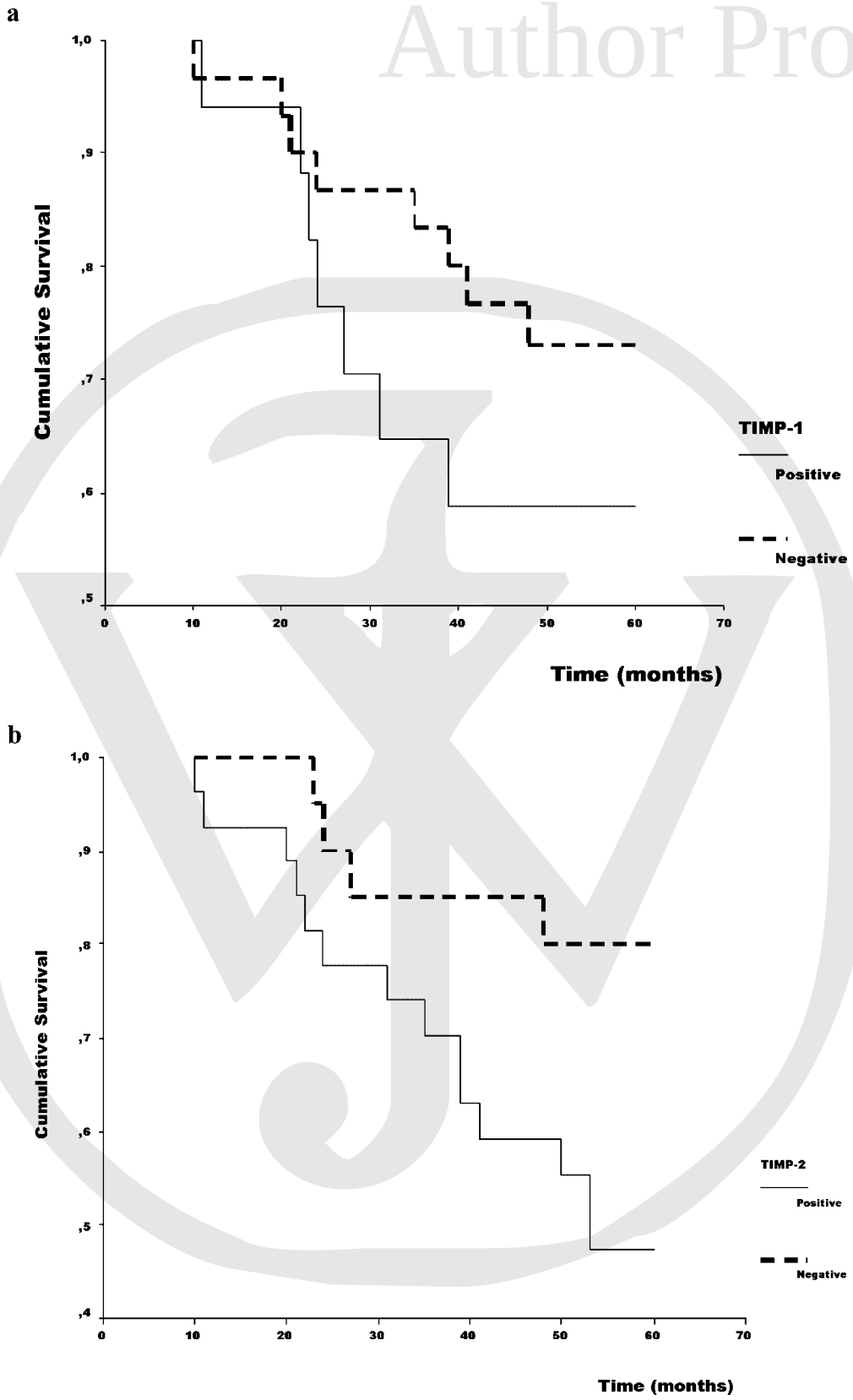

Fig. 2. Kaplan–Meier curves of overall survival categorized by TIMP-1 (**a**) and TIMP-2 (**b**) labeling index. Tumors were classified as positive when $\geq$25% of the cells were specifically stained. Difference in survival was only statistically significant for TIMP-2 (Log rank test 4.69, $P < 0.05$).

TABLE V. Cox Survival Model for TIMP-2 Overexpression in CRC Patients

Independent variable	Coefficient	*P*	Relative risk	95% confidence interval
TIMP-2	1.32	0.001	3.76	1.69–8.36
Sex	−0.912	0.20	0.40	0.10–1.62
Stage	1.19	0.01	3.28	1.63–6.63
Differentiation grade	0.17	0.76	1.18	0.41–3.39
Localization	0.36	0.30	1.43	0.73–2.77
Complication	0.06	0.92	1.06	0.35–3.27
Invasion	0.10	0.69	1.11	0.67–1.82

A new COX study replacing stage by the number of metastatic lymph nodes maintained TIMP-2 as an independent tumor marker and confirmed that the presence of metastatic lymph nodes is an independent prognostic marker [Coef 1.63, $P = 0.001$, RR: 5.08 (2.08–12.56)].

Reduced expression of both E-cadherin and β-catenin in carcinoma cells have been associated with dedifferentiation, increased invasiveness, and advanced stage in a number of tumors [14–17], including tumors of the digestive system [18–21]. We demonstrated a significant loss of E-cadherin and membrane β-catenin expression in our population of CRC tumors compared with normal colonic tissue. We found a high correlation between the expression of E-cadherin and β-catenin at membrane level in CRC cases. However, we did not find any relationship between decreased E-cadherin or β-catenin expressions and the main clinicopathological parameters in this population. Interestingly, survival analysis demonstrated that the subpopulation of patients with CRC tumor underexpressing E-cadherin had a worse evolution and furthermore the multivariate study demonstrate that this molecule could be considered an independent CRC prognosis marker. Other works have associated loss of E-cadherin with bad outcome in different tumoral pathologies, including CRC [19–23]. On the other hand, the prognostic value of β-catenin remains controversial [24]. According to our study β-catenin expression or accumulation in the nucleus did not correlate with survival in CRC patients.

Diverse mechanisms lead to β-catenin deregulation in tumors and its abnormal nuclear accumulation including mutations in NH_2-β-catenin sequences or alterations in the Wnt signaling pathway as well as inactivating mutations in the *APC*, *AXIN1*, and *AXIN2* genes [25–27]. Interestingly, associated with the decreased β-catenin expression in cell membranes, we observed a nuclear accumulation of β-catenin in 55% of tumor specimens, as Mikami et al. [28] have also found. This finding suggests that β-catenin deregulation is a common feature of CRC, although we still do not know the mechanisms involved in this deregulation in our CRC population.

MMPs play a key role in the infiltration and metastasis of several carcinomas. They are regulatory molecules, both by functioning in enzyme cascades and by processing matrix proteins, cytokines, growth factors, and adhesion molecules to generate fragments with enhanced or reduced biological effect [29]. Most MMPs are secreted as proenzymes into the ECM environment by stromal and tumoral cells [30]. Recently, it was understood the complex mechanism of activation of these latent forms to fulfill their proteolytic ability. It is known that TIMP-2 forms a complex with MT1-MMPs, an enzyme anchored to the plasma membrane and promotes the hydrolysis of pro-MMP-2 to its active form [31]. Besides, pro-MMP-9 can be activated by MMP-2, together with other enzymes as MMP-3 and MMP-13 [32]. In this sense, we found a significant coexpression between MMP-2 and TIMP-2 in our CRC samples. On the other side, we found a high correlation between MMP-9 and 2 expression suggesting that they may share some intracellular pathways able to regulate their synthesis, activation, and/or secretion.

Several authors have studied the expression of MMPs and TIMPs in human tumors employing different methodologies [33,34]. However, only few evaluated simultaneously the expression of several MMPs and TIMPs. In our study we found a very high expression of MMP-2, 7, and 9, possibly due to the fact that the employed antibodies recognize both latent and active MMPs forms. We observed specific MMPs staining not only in the cytoplasm of tumoral cells but also in the extracellular matrix of tumors, being both stainings highly correlated. On the other hand, our univariate studies indicated that no MMP was associated with the known clinico-pathological parameters in CRC.

MMP-7 (Matrylisin, pump-1) is the smallest known member of the MMP family, capable of degrading various ECM proteins, adhesion molecules as E-cadherin and also of activating pro-MMP-1, 2, and 9 [35,36]. MMP-7 is produced almost exclusively by epithelial cells, in particular by the glandular epithelium. When these cells have lost their polarity, MMP-7 is secreted through the luminal surface. The capacity of MMP-7 to degrade vascular basement membranes suggests its potential to facilitate hematogenous metastases. It has been reported that MMP-7 is overexpressed both in benign and malignant CRC [37]. We found an extremely

high expression of MMP-7 in our series of CRC, as almost 80% of the tumors presented more than 25% of positive cells. To emphasize the role of MMP-7 some authors demonstrated that the treatment of human colon carcinoma cells with active matrilysin promotes liver metastasis in nude mice through the modulation of the E-cadherin /β-catenin system [38]. On the other hand, it has been reported that the transcription of MMP-7 is regulated by β-catenin in colon cancer [39]. In the present study, we found a correlation between staining for nuclear β-catenin and the expression of MMP-7. Thus, it is possible that β-catenin could be regulating the enhanced secretion of this metalloproteinase.

Although an association between MMP-9 expression and survival has been reported by other authors [34,40], in the present series of CRC patients no association was found. As only a high expression of MMP-7 (in more than 50% of tumoral cells) showed a borderline significant association with poor survival, it would be important to extend the study to a higher number of patients.

We observed a high expression of TIMP-1 and 2, both in the cytoplasm of the tumoral cells and in the ECM, with no association with any of the prognostic markers in CRC pathology. Although early studies have shown that TIMPs have antitumor or antimetastatic effects, more recent reports indicate a dual function for these molecules, as TIMPs can directly affect cell growth and survival independently of their actions on MMPs [41,42]. High TIMPs expression in tumor tissue closely correlates with poor outcome in some human cancers [34,43]. With regard to gastrointestinal tumors, it was reported that increased TIMP-1/TIMP-2 mRNA levels correlated with tumor stage, lymph node metastasis and shortened survival in patients [44]. We found that both TIMP1 and 2 were associated with bad outcome in CRC but after multivariate analysis, only the overexpression of TIMP-2 could predict overall survival in an independent way. The paradoxical poor prognostic significance of TIMP-2 overexpression, as demonstrated in this study and others, warrants further investigation to fully understand the complex MMP/TIMP interactions, mainly taking into account that several pharmaceutical companies are currently developing low-molecular weight molecules to modulate MMPs for clinical use.

CONCLUSIONS

In this study, we demonstrate alterations in the E-cadherin/β-catenin system with concomitant loss of E-cadherin and β-catenin at membrane level and an abnormal accumulation of nuclear β-catenin. Besides, we found that all MMPs studied and both TIMPs were overexpressed in CRC tissue at protein level. The expression of these molecules was not associated with the known clinical and pathological parameters employed in CRC pathology. However, a multivariate analysis demonstrated that the overall survival could be predicted by the loss of E-cadherin and by the overexpression of TIMP-2. Besides, our results are validated by the fact that overall survival can be also predicted by the stage, the number of metastatic lymph nodes and the occurrence of vascular and/or neural invasion, indicating the representativity of the studied group.

A deeper understanding of the molecular genetic abnormalities involved in CRC pathogenesis, biological behavior, and therapeutic response may help to identify new targets for therapy and thereby improve patients' stratification and our ability to treat them successfully.

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We analyzed retrospectively the prognostic input of E-cadherin, β-catenin, MMPs (7 and 9), and TIMPs (1 and 2) in patients with colorectal carcinoma with a follow-up period of 5 years for survivors. The multivariate analysis demonstrated that the overall survival of patients could be independently predicted by the loss of E-cadherin and the overexpression of TIMP-2.

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