Prognostic Value of *LISCH7* mRNA in Plasma and Tumor of Colon Cancer Patients

José M. García,[1] Cristina Peña,[1] Vanesa García,[1] Gemma Domínguez,[1] Concepción Muñoz,[3] Javier Silva,[1] Isabel Millán,[2] Raquel Diaz,[1] Yolanda Lorenzo,[1] Rufo Rodriguez,[4] and Félix Bonilla[1]

Abstract

Purpose: *LISCH7* is a gene potentially regulated by p53 that is up-regulated in metastasis development. Our hypothesis was that the expression of *LISCH7* in primary colorectal tumors determined certain characteristics of the tumors, as well as their behavior, and that its identification in plasma could serve as a prognostic marker.

Experimental Design: We tested this hypothesis in a series of 115 tumors and normal tissues and in 83 plasmas from patients with sporadic colorectal carcinomas, as well as in 20 healthy control plasmas in which the expression levels of the gene were measured by real-time PCR. The expression data were contrasted with clinicopathologic variables.

Results: Although *LISCH7* expression was not detected in any control plasma samples, it was positive in 25 (30.1%) plasmas from patients ($P = 0.002$). *LISCH7* mRNA in plasma was significantly associated with the pathologic stage ($P = 0.019$), with lymph node metastasis ($P = 0.008$) and with vascular invasion ($P = 0.005$). Expression was not detected in any normal tissues but was detected in 80 tumor tissues, with a clear association found with vascular invasion ($P = 0.027$). Moreover, we show that *LISCH7* was specifically expressed by the epithelial tumor cells. The adjusted overall survival study showed independent prognostic values for *LISCH7* expression levels in tumor tissues (hazard ratio, 3.45; 95% confidence interval, 1.19-9.98).

Conclusions: Our results suggest that *LISCH7* is a good tumor marker whose expression levels could be considered as a poor prognosis factor in human colon cancer. Furthermore, plasma is suggested as a feasible source of nucleic acids for subsequent noninvasive prognostic studies.

Tumor metastases are the final consequence of tumor progression and the most common cause of death in cancer patients. The first step in the development of metastasis is invasion of the adjacent tissue; then, in the second step, intravasation, the neoplastic cells enter the blood or lymph vessels, and some survive to the next step, extravasation at distal sites, where a subset of such metastasizing cells will form a tumor metastasis (1).

Experimental animal models have been successfully used to identify molecular elements during metastasis in melanoma and breast cancer (2, 3). Recently, Yang et al. (4) have studied a mouse mammary tumor model, in which a set of otherwise isogenic tumor cell populations completed distinct steps of metastasis when implanted into the mammary glands of BALB/c mice. One of the most up-regulated genes related to development of visible metastasis was *LISCH7*, liver-specific bHLH-Zip transcription factor, which is involved in the regulation of transcriptional processes. In addition, Kannan et al. (5) have proposed that *LISCH7* could be a primary target of p53, although transactivation of *LISCH7* by stress conditions is not discarded.

The search for new methods to detect metastatic or recurrent disease at preclinical or presymptomatic stages may contribute to improved patient survival. Cancer patients have higher concentrations of circulating nucleic acids in plasma and serum, and patients with metastasis have still higher levels (6, 7). Moreover, tumor alterations in DNA and RNA have been detected in plasma of patients with various types of cancer (8–18) and are associated with poor prognosis (14–17, 19–22). Nevertheless, mRNA-based amplification methods can offer greater specificity and sensitivity than DNA alteration detection (23). These data suggest the possibility of developing a noninvasive diagnostic and prognostic method by measurement of tumor RNA in plasma, which would be useful during the follow-up of patients.

Based on such evidence, we studied the presence of the *LISCH7* mRNA in plasma from colorectal patients and healthy blood donors to examine its specificity for the disease and to search its possible prognosis value. Finally, the expression levels

Authors' Affiliations: Departments of [1]Medical Oncology and [2]Biostatistics, Hospital Universitario Puerta de Hierro, Madrid, Spain and Departments of [3]Gastroenterology and [4]Pathology, Hospital Virgen de la Salud, Toledo, Spain

Received 4/13/07; revised 7/18/07; accepted 7/27/07.

Grant support: SAF2004-01002 and FIS-PI040053 and Fundación MMA.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

J.M. García and C. Peña contributed equally to this work.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Félix Bonilla, Department of Medical Oncology, Hospital Universitario Puerta de Hierro, C/San Martín de Porres, 4, E-28035 Madrid, Spain. Phone: 34-91-3445148; Fax: 34-91-3445189; E-mail: fbonilla.hpth@salud.madrid.org.

doi:10.1158/1078-0432.CCR-07-0882

of this gene in primary tumors were also studied to infer a possible relationship with survival or the behavior of the disease, as well as its cellular origin.

Materials and Methods

Patients, samples, and RNA extraction. The study was approved by the research ethics board of our hospital and informed consent was obtained from all participants.

Between January 2005 and June 2006, blood samples (20 mL) were taken from 83 patients with colorectal carcinoma by venipuncture before intervention on the day of surgery. Blood samples from 20 healthy blood donors were also obtained at the hematology unit of our hospital. Plasma was prepared by centrifugation of peripheral blood at 2,500 rpm for 25 min and snap frozen at -80°C until processing. Tissue samples were also obtained from 20 of the colorectal carcinoma patients immediately after surgery, immersed in RNA later (Ambion, Inc.), snap frozen in liquid nitrogen, and stored at -80°C until processing. Normal tissues were taken from a noncancerous region of the colon mucosa at least 3 cm from the outer tumor margin.

Between June 1999 and December 2000, we recruited 95 patients diagnosed with colorectal cancer. They were included in a prospective study to evaluate overall survival (OS) and disease-free survival (DFS). Tissue samples from these patients were obtained, as mentioned above.

RNA of plasma samples was obtained from 1 mL of plasma by the Dynabeads mRNA DIRECT kit (Dynal Biotech ASA). RNA from tissue samples was extracted from ~30 mg of tumor and normal samples, using RNeasy Mini kit (Qiagen, Inc.).

Epithelial cell selection/purification. Normal and tumor fresh samples from five patients were disaggregated in a Medimachine instrument (DAKOCytomation). Cell viability was evaluated by trypan blue exclusion, all (>95%) of the cells in this fraction being viable. Epithelial cells were immunomagnetically purified from 2×10^6 total cells using superparamagnetic polystyrene beads coated with the Ber-EP4 antibody (Dynabeads Epithelial Enrich, Dynal Biotech ASA). mRNA was extracted from the epithelial cell–enriched fraction (>97%) and from the supernatant containing nonepithelial cells. Cellular nuclei were labeled with 4′,6-diamidino-2-phenylindole (Sigma). Monoclonal antibodies against cytokeratin 8, 18, 19, conjugated with FITC, were used to label and to visualize epithelial cells specifically (DAKOCytomation) under the fluorescence microscope.

Real-time PCR. Because *LISCH7* mRNA was not detected in plasma of healthy donors, or in normal tissue from patients, two methods for expression analysis were used in the study. First, *LISCH7* mRNA expression was measured only in plasma and tumor tissue samples in a relative quantification in samples in which its detection was possible. When *LISCH7* expression was not detected in tumor tissues, half the minimum value detected in the series was assigned (this value was 0.01). Second, as there were some patients in whom *LISCH7* mRNA in plasma was not detected, *LISCH7* expression in plasma was also evaluated as present or absent.

LISCH7 mRNA expression in each sample was measured as a ratio against the geometric average of three reference housekeeping genes, *succinate dehydrogenase complex subunit A, TATA binding protein*, and *ubiquitin C* (24). The relative concentrations of the target and the

Table 1. Association of clinicopathologic characteristics among qualitative and quantitative *LISCH7* mRNA data in plasma and quantitative *LISCH7* mRNA data in tumors

Characteristics	Total (%), plasma samples	*LISCH7* mRNA in plasma					Total (%), tumor samples	*LISCH7* expression in tumors	
		Presence			Relative amount			Median/minimum/maximum (relative amount)	*P**
		No	Yes	*P* †	Geometric average	*P* ‡			
Patients	83	—	—	—	—		115	—	
Median age (y)	70 ± 11				—		70 ± 11	—	
Sex									
Male	49 (59)	35	14	NS	2	NS	72 (62.6)	0.89/0.01/28	NS
Female	34 (41)	23	11		1.2		43 (37.4)	1.22/0.01/28	
Tumor side									
Right	28 (33.7)	18	10	0.103	1.2	NS	34 (29.6)	0.66/0.01/8.84	NS
Left-sigma	34 (41)	28	6		1.1		37 (32.2)	1.15/0.01/28	
Rectum	21 (25.3)	12	9		2.5		44 (38.2)	1.05/0.01/28	
Stage									
I	7 (8.5)	6	1		0.5		12 (10.4)	0.67/0.01/4	
II	31 (37.3)	26	5	0.019	0.4	0.056	55 (47.8)	0.79/0.01/28	NS
III	23 (27.7)	16	7	0.012§	2.7	0.005§	42 (36.5)	1.01/0.01/7.94	NS§
IV	22 (26.5)	10	12		2.3		6 (5.2)	0.89/0.01/28	
Vascular invasion									
Yes	25 (30.1)	12	13	0.005	1	NS	45 (39.1)	0.89/0.01/28	0.027
No	58 (69.9)	46	12		2.5		70 (60.9)	0.21/0.01/14	
LNM									
Positive	45 (54.2)	26	19	0.008	2.5	0.005	45 (39.1)	0.89/0.01/28	NS
Negative	38 (45.8)	32	6		0.4		70 (60.9)	1.11/0.01/28	
Tumor differentiation									
Well	30 (36.2)	24	6	NS	0.6	NS	33 (28.7)	0.45/0.01/14	NS
Moderate	43 (51.8)	28	15		2.7		61 (53.0)	1.37/0.01/8.84	
Poor	10 (12)	6	4		0.9		21 (18.3)	0.89/0.01/28	

Abbreviation: NS, not significant.
*P was calculated by Kruskal-Wallis test.
†P was calculated by χ^2 test.
‡P was calculated by ANOVA test with positive *LISCH7* expression cases.
§Statistical analysis of early stages (I + II) relating to advanced stages (III + IV).

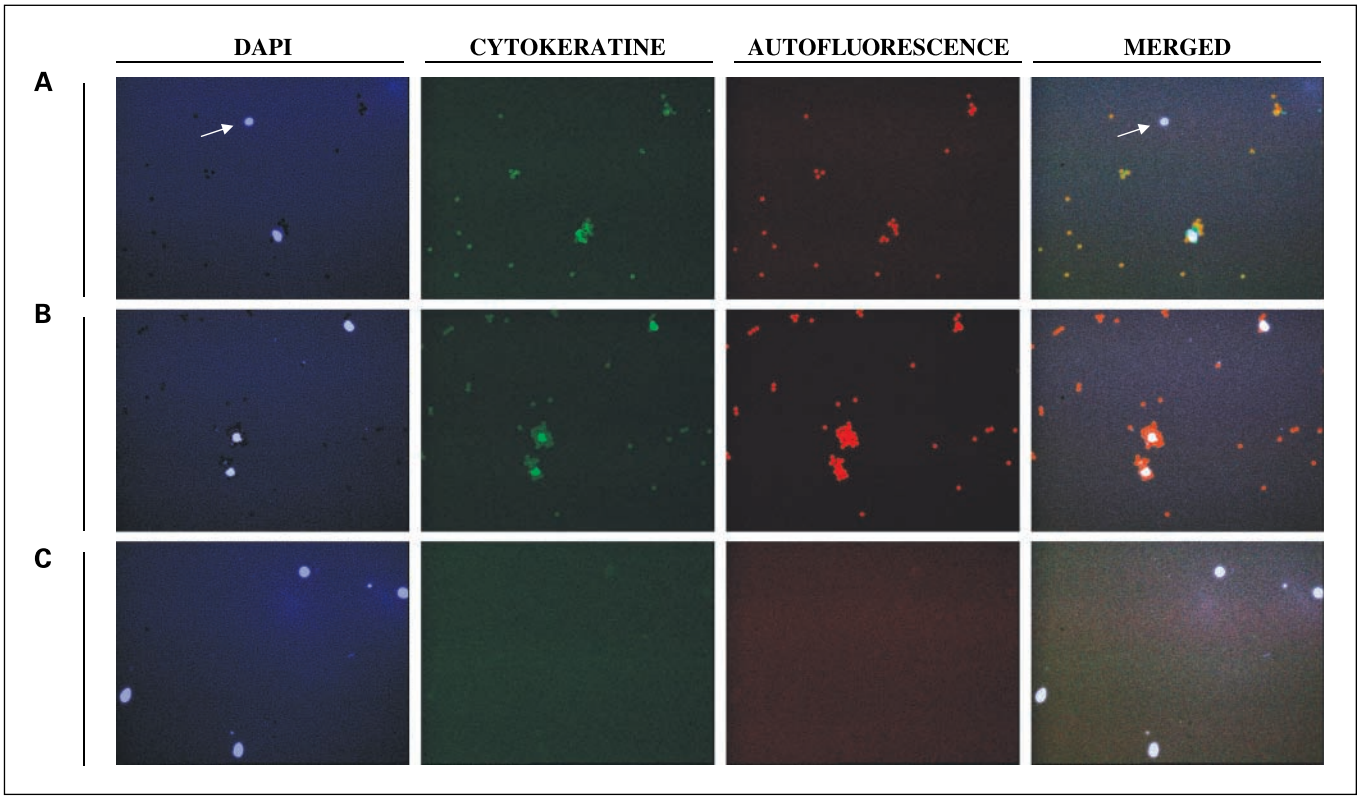

Fig. 1. Images under the fluorescence microscope of the different cellular fractions. *A,* whole disaggregated sample in which a nonepithelial cell [4′,6-diamidino-2-phenylindole (*DAPI*) positive and cytokeratin (*CK*) negatives; *arrow*] and an epithelial cell (4′,6-diamidino-2-phenylindole and cytokeratin positives) are shown. The nonepithelial cell is not joined to the magnetic beads. *B,* epithelial-enriched fraction where all the cells observed were epithelial cells. *C,* supernatant fraction where epithelial cells were not observed.

reference genes were calculated by interpolation using a standard curve of each gene plotted from the same serial dilution of cDNA from tumor tissue. The quantitative mRNA analysis was done in duplicate. Primers are shown in Supplementary Table (Supplementary Materials and Methods). For the synthesis of cDNA, RNA was retrotranscribed using the Gold RNA PCR Core kit (PE Biosystems).

Real-time PCR was done in a LightCycler apparatus (Roche Diagnostics) using the LightCycler FastStart^{PLUS} DNA Master SYBR Green I kit (Roche Diagnostics).

Clinicopathologic variables. Clinicopathologic variables, listed in Table 1, were obtained from the medical records of the 178 patients. Pathologic stage was assessed using the tumor-node-metastases classification. Presence of lymph node metastases (LNM) was evaluated by optical microscopy.

Patient follow-up. Clinical follow-up after surgery and diagnosis was based on periodic visits and biochemical and imaging techniques (chest X-ray, endoscopy, bone scan, and other areas as clinically indicated). Ultrasonic study was done when liver function was impaired. OS and DFS were defined as the period from time of diagnosis until death and the interval between diagnosis and first recurrence, respectively.

Statistical analysis. Association between presence or absence of *LISCH7* mRNA in plasma and presence or absence in healthy donors and clinicopathologic variables of the tumor or *LISCH7* expression in tumors were contrasted by the χ^2 test.

LISCH7 mRNA data in plasma was not normally distributed (Kolmogorov-Smirnov test, Lilliefors correction). We standardized data distribution by using $\log_{10}$ for statistical analysis and we also used the geometric average to describe expression gene data and association between *LISCH7* mRNA in plasma and clinicopathologic characteristics were contrasted by the one-way ANOVA test.

LISCH7 expression data in tumors were not normally distributed (Kolmogorov-Smirnov test), although $\log_{10}$ was used, so the geometric average was used to describe the gene expression, and the Kruskal-Wallis test was applied to contrast expression data with clinicopathologic variables.

To study OS and DFS, the expression data of *LISCH7* were divided by tertiles. The expression levels defining the three groups were 0.05 (33%) and 2.24 (66%). The relationship between the cumulative probability of OS and DFS, as well as analyzed predictors, was calculated following the Kaplan-Meier method (25), whereas significant differences between curves were evaluated with Mantel's log-rank test (26). To identify factors that might be of independent significance in influencing OS and DFS, multivariate analysis (Cox proportional risk regression model) was applied (27). 'Confuser' and interacting variables were analyzed. The model's basic assumptions (proportional hazards) were evaluated.

In all statistical tests, two-tailed $P \leq 0.05$ was considered statistically significant. Statistical analysis was done using version 11.0 of the SPSS.

Results

LISCH7 expression in plasma and tumor samples. Of the 83 plasma samples analyzed from colorectal cancer patients, 25 (30.1%) showed presence of *LISCH7* mRNA. However, it was not detected in any of the 20 plasma samples from healthy donors (P = 0.002). The quantification of *LISCH7* mRNA in plasma showed a median of 1.45 (minimum, 0.05; maximum, 9.55).

There were 115 tumor and normal counterpart tissue samples. *LISCH7* expression was not detected in any normal samples. However, 80 (69.6%) adenocarcinomas showed

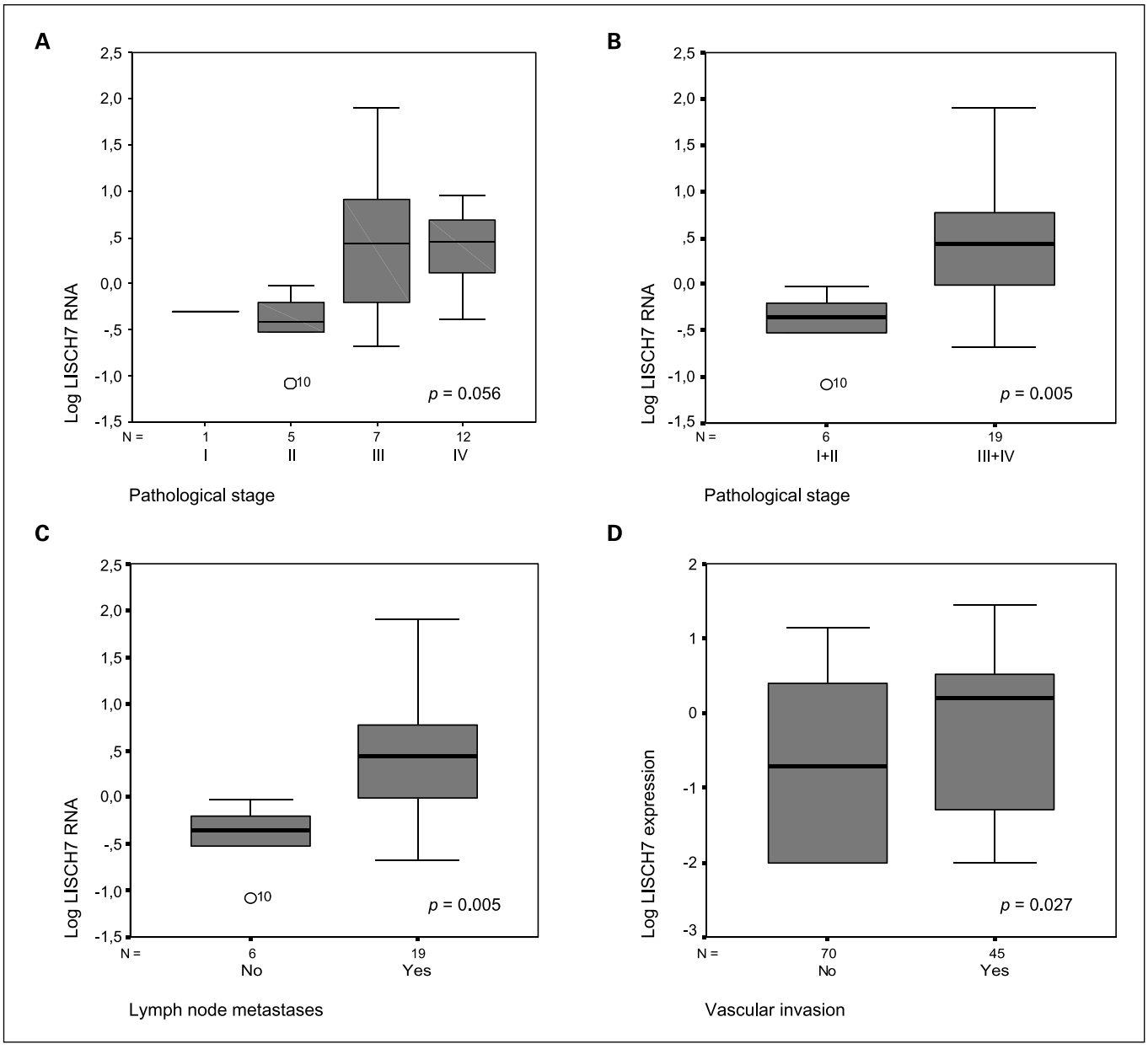

Fig. 2. Box plots showing relationships between some tumor characteristics and relative amounts of *LISCH7* mRNA in plasma or in tumor samples. *A,* pathologic stage and *LISCH7* mRNA in plasma. *B,* pathologic stage grouped in early and advanced stages and *LISCH7* mRNA in plasma. *C,* LNM and *LISCH7* mRNA in plasma. *D,* vascular invasion and *LISCH7* expression in tumors. Circles represent outliers.

LISCH7 expression. The quantification of *LISCH7* expression in tumor tissue showed a median expression of 2.46 (minimum, 0.02; maximum, 28.00).

There were 20 colorectal cancer patients in whom tumor and plasma samples were available. Among these 20 patients, only 1 was a stage I patient and both plasma and tumor tissue were *LISCH7* mRNA negative; 10 were patients at stage II, in 7 of whom *LISCH7* mRNA was detected in tissues samples and 2 plasmas of these showed positive presence of the target gene; 3 cases of the 8 patients at stage III had *LISCH7* mRNA in tumors and 2 of them also had it in plasma. Finally, there was 1 patient at stage IV, and *LISCH7* mRNA was only detected in the plasma sample. In other words, of the 5 patients with LISCH7 mRNA in plasma, 4 also showed it in tumor tissues.

LISCH7 cellular expression origin. We tested the possibility that the expression of *LISCH7* could correspond to stromal cells rather than carcinoma cells in the biopsies. Nonepithelial cytokeratin-negative cells constituted 5% to 15% of the whole tumor sample disaggregates but were absent in epithelial-enriched fractions following separation with paramagnetic beads (Fig. 1). *LISCH7* mRNA was detected specifically in epithelial fraction from three of the five tumoral samples (data not shown).

LISCH7 mRNA and pathologic data in plasma samples. Presence of *LISCH7* mRNA in plasma was associated with stage, LNM, and vascular invasion (Table 1). A difference toward statistical significance was observed for the tumor side because the presence of *LISCH7* mRNA was greater in patients with a rectum cancer.

In the plasma samples in which *LISCH7* mRNA was detected, the amounts of it were contrasted with pathologic variables, and some associations were found. Thus, it was associated with presence of LNM (Fig. 2C; Table 1). However, no association was found with vascular invasion. For the stage, a difference close to statistical significance was found ($P = 0.056$), but this difference was clearly significant when the stages were grouped in early (I + II) and advanced (III + IV) stages ($P = 0.005$; Fig. 2A and B; Table 1).

***LISCH7* expression and pathologic data in tumor samples.** In tumors, an association between up-regulation of *LISCH7* expression and vascular invasion was observed (Fig. 2D; Table 1). Although no association was found between *LISCH7* expression and stage, a similar scenario to those for plasma

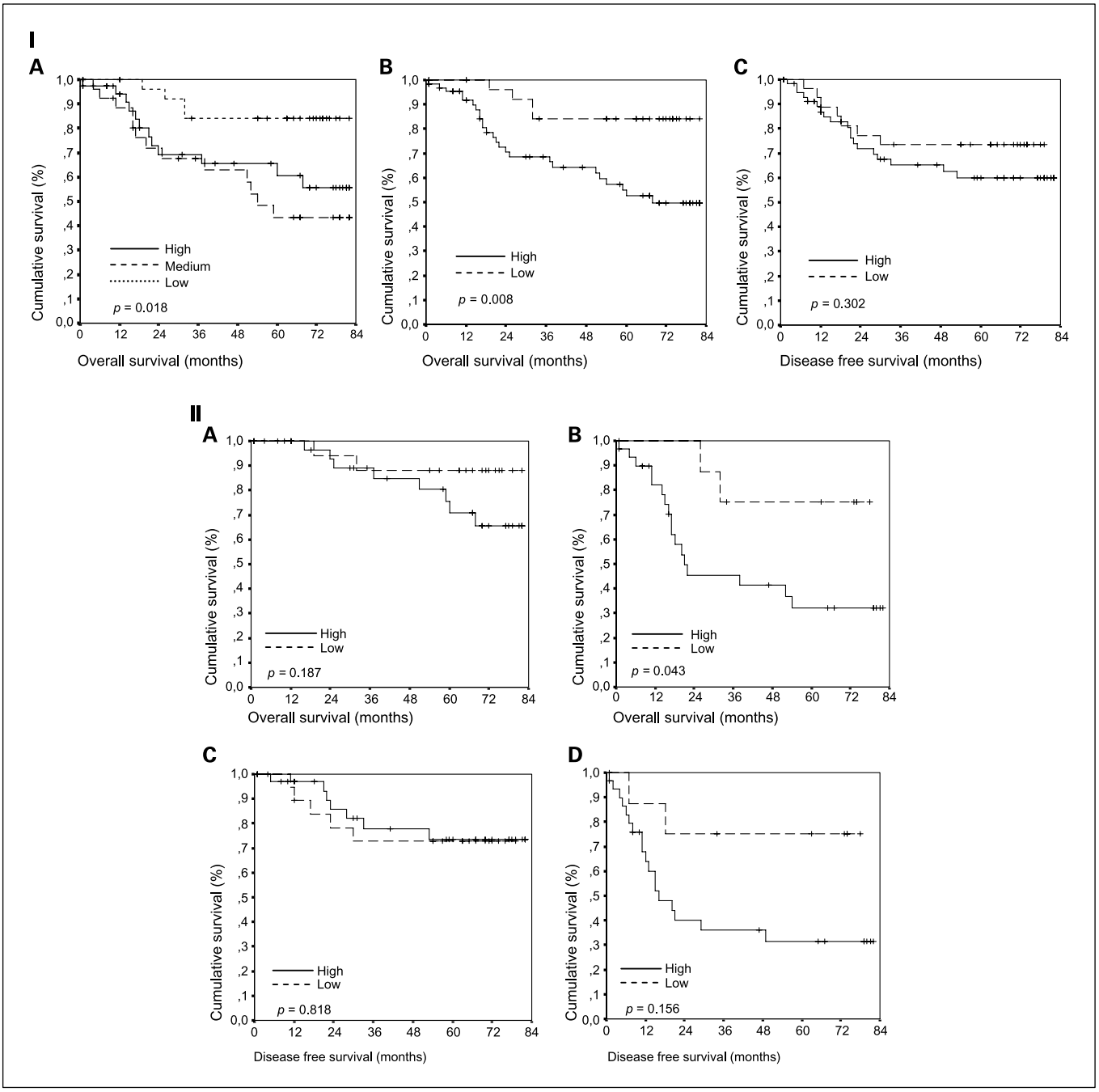

Fig. 3. *I,* influence of colon carcinoma mRNA levels of *LISCH7* gene on OS and DFS, Kaplan-Meier curves, and *P* values. *A, LISCH7* expression in tumors distributed by tertiles: low, showing a 5-y OS rate of 84% (95% CI, 70-98%); medium, 43% (95% CI, 23-63%); and high, 61% (95% CI, 43-79%). *B,* comparison between high *LISCH7* expressions, taking as high expression, medium and high subgroups with a 5-year OS rate of 53% (95% CI, 39-67%), and low, with a rate of 84% (95% CI, 70-98%). *C,* low expression levels with 5-y DFS of *LISCH7* analysis. Low levels, 73% (95% CI, 55-91%); high levels, 60% (95% CI, 46-74%). *II,* Kaplan-Meier OS (*A* and *B*) and DFS (*C* and *D*) curves analyzed by stages. *A,* no difference in stages I + II for *LISCH7* expression. *B,* difference in stages III + IV and *LISCH7* mRNA level: low *LISCH7* expression levels with a 5-year OS rate of 75% (95% CI, 46-100%); high, 32% (95% CI, 12-51%). *C,* no difference between *LISCH7* expressions at stages I + II. *D,* tendency to significance in *LISCH7* expressions at stages III: low, 75% (95% CI, 46-100%); high, 41% (95% CI, 19-63%).

Table 2. Univariate and multivariate analyses of the association between *LISCH7* expression and OS (A) as well as DFS (B) of colon cancer patients with a median follow-up of 47 mo

Variable	Category	Unadjusted analysis		Adjusted analysis	
		HR (95% CI)	*P*	HR (95% CI)	*P*
A.					
Age at diagnosis (y)	<71 vs ≥71	1.83 (0.83-4.02)	0.132		
Sex of patients	Male vs female	2.25 (0.96-5.27)	0.062	2.28 (0.97-5.36)	0.058
LNM	Yes vs No	3.20 (1.53-6.73)	0.002		
Vascular invasion	Yes vs No	2.46 (1.18-5.12)	0.017		
Stage	I + II vs III + IV	3.70 (1.71-7.96)	0.001	3.45 (1.60-7.51)	0.002
Histologic grade	2 vs 1	1.91 (0.76-4.77)	0.167		
	3 vs 1	0.79 (0.20-3.17)	0.741		
Tumor side	Left-sigma vs right	1.48 (0.53-4.17)	0.456		
	Rectum vs right	1.81 (0.70-4.72)	0.224		
LISCH7 expression	High vs low	3.80 (1.32-10.93)	0.013	3.45 (1.19-9.98)	0.022
B.					
Age at diagnosis (y)	<71 vs ≥71	1.68 (0.75-3.76)	0.211		
Sex of patients	Male vs female	2.51 (1.01-6.26)	0.048	2.77 (1.11-6.93)	0.030
LNM	Yes vs No	2.20 (1.02-4.77)	0.045		
Vascular invasion	Yes vs No	2.32 (1.07-5.02)	0.032		
Stage	I + II vs III	2.50 (1.15-5.41)	0.020	2.73 (1.27-5.94)	0.011
Histologic grade	2 vs 1	2.04 (0.76-5.47)	0.156		
	3 vs 1	0.62 (0.12-3.20)	0.567		
Tumor side	Left-sigma vs right	0.91 (0.34-2.44)	0.858		
	Rectum vs right	1.02 (0.40-2.39)	0.965		
LISCH7 expression	High vs low	1.57 (0.66-3.74)	0.308		

NOTE: The blank cells correspond to variables that showed no independent relations to OS in the multivariate analysis.

LISCH7 mRNA was observed. Therefore, there were 7 (58%) patients with *LISCH7* expression at stage I, 39 (71%) with it at stage II, 29 (69%) with it at stage III, and, again, the highest proportion of patients with *LISCH7* expression was at stage IV, 5 (83%).

Overall survival. The 5-year OS for patients was 63% [95% confidence interval (95% CI), 52-74%].

Patients were divided into tertiles based on *LISCH7* expression. When OS was analyzed for *LISCH7* expression, a significant difference was observed ($P = 0.018$; Fig. 3I, *A*). The Kaplan-Meier graph revealed similar behavior of the medium- and high-level tertiles. Thus, these patients were grouped as a high expression group and additional analysis of *LISCH7* expression was carried out with only two categories, low- and high-expression levels of *LISCH7*. In this analysis, the significant difference is clearer than in the previous one ($P = 0.008$; Fig. 3I, *B*).

The univariate analysis showed that variables that could be regarded as statistically supported factors in OS prediction were sex of patients, vascular invasion, LNM, stage (III and IV versus I and II), and *LISCH7* expression. Multivariate analysis showed that sex, stage, and *LISCH7* expression [hazard ratio (HR), 3.45; 95% CI, 1.19-9.98] had independent relations to OS (Table 2A).

The correlation between overall survival and *LISCH7* expression was analyzed separately in stages I + II and in III + IV. A significant difference was found for *LISCH7* expression at stages III + IV ($P = 0.043$), but not at stages I + II (Fig. 3II, *A* and *B*).

The multivariate analysis with the series stratified according to stage confirmed that *LISCH7* expression had clearer independent relations to OS at stages III + IV (HR, 3.63; 95% CI, 0.83-15.89; Table 3A) than at stages I + II.

Disease-free survival. During follow-up, 32 (33.7%) relapses were observed.

Table 3. Analysis of the association between *LISCH7* expression and OS (A) as well as DFS (B) in colon cancer patients regarding pathologic stages

Variable	Category	Multivariate analysis (stages I + II)		Multivariate analysis	
		HR (95% CI)	*P*	HR (95% CI)	*P*
A.				Stages III + IV	
Sex of patients	Male vs female	1.91 (0.49-7.50)	0.352	2.46 (0.81-7.47)	0.112
LISCH7 expression	High vs low	2.99 (0.63-14.24)	0.169	3.63 (0.83-15.89)	0.087
B.				Stage III	
Sex of patients	Male vs female	2.04 (0.55-7.60)	0.290	3.36 (0.91-12.34)	0.068
LISCH7 expression	High vs low	0.95 (0.30-3.03)	0.934	2.42 (0.54-10.92)	0.251

No clear differences in DFS for *LISCH7* expression were observed (Fig. 3I, *C*).

The univariate analysis showed that sex, vascular invasion, LNM, and stage could be regarded as statistically supported factors in DFS. Multivariate analysis showed that sex and stage had independent relations with DFS (Table 2B).

Although the Kaplan-Meier test revealed no difference in DFS for *LISCH7* expression in stage groups, the same tendency that *LISCH7* expression showed better OS prognosis value in advanced than in early stages was seen (Fig. 3II, *C* and *D*). The multivariate analysis according to stage did not confirm this fact. However, the same tendency could be observed. Thus, *LISCH7* expression had a HR of 0.95 (95% CI, 0.30-3.03; $P = 0.921$) at stages I + II and 2.42 (95% CI, 0.54-10.92; $P = 0.251$) at stage III (Table 3B).

Discussion

The presence of *LISCH7* mRNA in plasma from patients with colorectal cancer was studied to validate its disease-predictive value because it could then be used as a noninvasive tool in prognosis. An association between the presence of extracellular plasma RNA of specific genes and poor prognosis has been reported in some cancer types (14, 16, 17). Our results are consistent with these findings because *LISCH7* mRNA in plasma was associated with presence of LNM, advanced stages, and vascular invasion, as well as a trend to tumor side in rectum, all of which suggest that it could be used as a prognosis factor.

LISCH7 mRNA was detected in 30.1% of plasmas from cancer patients. However, neither plasma from healthy control nor normal tissue samples showed the presence of *LISCH7* mRNA. These results suggest that *LISCH7* mRNA detected in plasma from patients with CC could have its origin in epithelial tumor cells, as it was observed with RNA isolated from the disaggregate tumor tissue and thus could be a specific tumor marker.

Interestingly, among patients with tumor and plasma samples, it was observed that *LISCH7* mRNA was detected in plasma from a cancer patient in whom expression of *LISCH7* was not detected in tumor tissue. We showed in a previous study that the presence of heterogeneous clones in the tumor samples could explain some nonmatched alterations between plasma and tumor (28). Additionally, in the current series, we observed that the detection of *LISCH7* in plasma is increasingly related to the presence of *LISCH7* in advanced-stage tumors. Thus, the *LISCH7* mRNA detected in plasma may be related to metastases because the up-regulation of this gene has been documented in cells able to develop metastasis, at least in animal models (4).

The follow-up of plasma samples was too short to carry out a survival study. However, our results in a long series of human colon cancer support the previous results because expressions of *LISCH7* were associated with a poor outcome of the disease and with more aggressive pathologic characteristics.

The finding that high levels of *LISCH7* predicted OS but not DFS in patients suggests that this gene could specifically confer advantage on metastasis enlargement (4). In fact, *LISCH7* expression levels are more clearly associated with poor prognosis for OS in advanced stages than in early stages, and the same tendency was observed for DFS. Therefore, for advanced stages (stage III in this case), 5-year DFS ran at 75% in patients with low *LISCH7* expression levels versus 40% in patients with high levels. This marked difference between the two groups was not statistically significant, probably due to the insufficient number of patients included in the study.

An association between the presence of *LISCH7* mRNA in plasma and advanced stages was found, but not clearly between the *LISCH7* expression in tumors and advanced stages. This might be due to the distribution of the patients in the series, as the plasma series contained proportionally more patients at stage IV than the tumor series did. However, it might also be because *LISCH7* mRNA in plasma is detected mainly at advanced stages. In this context, the detection of *LISCH7* mRNA in plasma could be a better prognostic tool than tumor samples because plasma data could be the surrogate for the *LISCH7* mRNA of primary tumor as well as of the metastatic tumor cells. Thus, hypothetically, it could have a possible clinical use as a serologic marker during the outcome of the patients.

Given the association between *LISCH7* mRNA in plasma and several poor prognosis factors and because *LISCH7* expression in tumors clearly influences disease outcome and given the high association between positive plasma and tumors, the value of *LISCH7* mRNA in the plasma of colorectal cancer patients can be considered a marker of poor prognosis and one that can be obtained by a noninvasive method.

Acknowledgments

We thank M. Eaude for help with the English manuscript and I. Alonso for blood sample collection.

References

1. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer 2003;3:453–8.
2. Clark EA, Golub TR, Lander ES, Hynes RO. Genomic analysis of metastasis reveals an essential role for RhoC. Nature 2000;406:532–5.
3. Kang Y, Siegel PM, Shu W, et al. A multigenic program mediating breast cancer metastasis to bone. Cancer Cell 2003;3:537–49.
4. Yang J, Mani SA, Donaher JL, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 2004;117:927–39.
5. Kannan K, Kaminski N, Rechavi G, Jakob-Hirsch J, Amariglio N, Givol D. DNA microarrays identification of primary and secondary target genes regulated by p53. Oncogene 2001;20:2225–34.
6. Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res 1976;37:646–50.
7. Shapiro B, Chakrabarty M, Cohn EM, Leon SA. Determination of circulating DNA levels in patients with benign or malignant gastrointestinal disease. Cancer 1983;51:2116–20.
8. Anker P, Lefort F, Vasioukhin V, et al. K-ras mutations are found in DNA extracted from the plasma of patients with colorectal cancer. Gastroenterology 1997;112:1114–20.
9. Silva JM, Gonzalez R, Dominguez G, Garcia JM, España P, Bonilla F. TP53 gene mutations in plasma DNA of cancer patients. Genes Chromosomes Cancer 1999;24:160–1.
10. Chen XQ, Stroun M, Magnenat JL, et al. Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat Med 1996;2:1033–5.
11. Lo YM, Chan LY, Lo KW, et al. Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma. Cancer Res 1999;59:1188–91.
12. Kopreski MS, Benko FA, Kwak LW, Gocke CD. Detection of tumor messenger RNA in serum of patients with malignant melanoma. Clin Cancer Res 1999;5:1961–5.
13. Chen XQ, Bonnefoi H, Pelte MF, et al. Telomerase RNA as a detection marker in the serum of breast cancer patients. Clin Cancer Res 2000;6:3823–6.
14. Silva JM, Dominguez G, Silva J, et al. Detection of epithelial messenger RNA in the plasma of breast cancer patients is associated with poor prognosis tumor characteristics. Clin Cancer Res 2001;7:2821–5.

15. Dasi F, Lledo S, Garcia-Granero E, et al. Real-time quantification in plasma of human telomerase reverse transcriptase (hTERT) mRNA: a simple blood test to monitor disease in cancer patients. Lab Invest 2001; 81:767–9.

16. Silva JM, Rodriguez R, Garcia JM, et al. Detection of epithelial tumor RNA in the plasma of colon cancer patients is associated with advanced stages and circulating tumour cells. Gut 2002;50:530–4.

17. Wong SC, Lo SF, Cheung MT, et al. Quantification of plasma β-catenin mRNA in colorectal cancer and adenoma patients. Clin Cancer Res 2004;10:1613–7.

18. Lo KW, Lo YM, Leung SF, et al. Analysis of cell-free Epstein-Barr virus-associated RNA in the plasma of patients with nasopharyngeal carcinoma. Clin Chem 1999;45:1292–4.

19. Silva JM, Dominguez G, Garcia JM, et al. Presence of tumor DNA in plasma of breast cancer patients: clinicopathological correlations. Cancer Res 1999;59: 3251–6.

20. Silva JM, Silva J, Sanchez A, et al. Tumor DNA in plasma at diagnosis of breast cancer patients is a valuable predictor for disease-free survival. Clin Cancer Res 2002;8:3761–6.

21. Taback B, Fujiwara Y, Wang HJ, Foshag LJ, Morton DL, Hoon DS. Prognostic significance of circulating microsatellite markers in the plasma of melanoma patients. Cancer Res 2001;61:5723–6.

22. Shao ZM, Wu J, Shen ZZ, Nguyen M. p53 mutations in plasma DNA and its prognostic value in breast cancer patients. Clin Cancer Res 2001;7:2222–7.

23. Silva J, Silva JM, Garcia V, Garcia JM, Dominguez G, Bonilla F. RNA is more sensitive than DNA in identification of breast cancer patients bearing tumor nucleic acids in plasma. Genes Chromosomes Cancer 2002; 35:375–6.

24. Vandesompele J, de Preter K, Pattyn F, et al. Accurate normalization or real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 2002;3:research0034.1.

25. Kaplan EL, Meier P. Non-parametric estimation from incomplete observations. J Am Stat Assoc 1958;53: 457–81.

26. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observations of each patients: II. Analysis and examples. Br J Cancer 1977;35:1–39.

27. Cox DR. Regression models and life tables. JR Stat Soc B 1972;34:187–20.

28. Garcia JM, Silva JM, Dominguez G, Silva J, Bonilla F. Heterogeneous tumor clones as an explanation of discordance between plasma DNA, tumor DNA. alterations. Genes Chromosomes Cancer 2001;31: 300–1.