Independent prognostic value of fascin immunoreactivity in stage III–IV colonic adenocarcinoma

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Fascin, an actin-bundling protein involved in cell motility, has been shown to be upregulated in several types of carcinomas. In this study, we investigated the expression of fascin in 228 advanced colonic adenocarcinoma patients with a long follow-up. Fascin expression was compared with several clinicopathologic parameters and survival. Overall, fascin immunoreactivity was detected in 162 (71%) tumours with a prevalence for right-sided tumours (P < 0.001). Fascin correlated significantly with sex, tumour grade and stage, mucinous differentiation, number of metastatic lymph nodes, extranodal tumour extension, and the occurrence of distant metastases. Patients with fascin-expressing tumours experienced a shorter disease-free and overall survival in comparison with those with negative tumours, and fascin immunoreactivity emerged as an independent prognostic factor in the multivariate analysis. Moreover, patients with the same tumour stages could be stratified in different risk categories for relapse and progression according to fascin expression. Our findings suggest that fascin is a useful prognostic marker for colonic adenocarcinomas.

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Colorectal cancer is among the most commonly diagnosed malignancies, ranking fourth in frequency in men and third in women (Parkin et al., 2005). Although the prognosis has slightly improved in the last few years (survival estimates at 5 years are now 65% in North America and 54% in Western Europe) (Parkin et al., 2005), colorectal cancer is still a major cause of death worldwide.

The recent introduction of new chemotherapeutic combinations of adjuvant chemotherapy has improved the disease-free survival (DFS) of stage III patients (Gramont, 2005). Nevertheless, a consistent fraction of patients with locally advanced colorectal carcinoma will relapse (Obrand and Gordon, 1997; Gramont, 2005), mostly within the first 3 years (Sadahiro et al., 2003). One of the reasons accounting for this failure may be the difficulty in stratifying patient groups with locally advanced disease in different risk categories. The issue of prognostic heterogeneity for patients with stage III colorectal cancer (Merkel et al., 2001) has, in part, been addressed by the last TNM edition, where colorectal cancer patients are substaged according to the depth of the intestinal wall invasion and the number of lymph nodes involved (Greene et al., 2002). In this scenario, a large number of biomolecular markers have been suggested as new prognostic factors in locally advanced colorectal carcinomas, but there are insufficient data or a poor clinical translation for most of them (Duffy et al., 2003; Funaioli et al., 2006).

The interaction among cancer cells, extracellular matrix proteins, and endothelial cells plays a major role in local invasion and distant metastases (Azzavoorian et al., 1993; Alessandro et al., 1996; Liotta and Kohn, 2001). Invasive tumour cells are often characterised by changes in cell shape with the appearance of membrane protrusions, loss of anchorage dependency, and loss of cell–cell adhesion and junctional communications. Many of these changes are related to re-arrangements of the cytoskeletal microfilaments because of the action of several types of actin cross-linking proteins (Maekawa et al., 1988; Tilney et al., 1998).

Among these molecules, fascins are involved in the organisation of two major forms of actin-based structures that include cortical cell protrusions such as filopodia, spikes, lamellipodial ribs, dendrites and microvilli, and cytoplasmic microfilament bundles (Kureishy et al., 2002). Fascin, which is expressed in normal mesenchymal, endothelial, dendritic, and neuronal cells but not in normal epithelia (Kureishy et al., 2002) is important in cell–cell interactions and adhesion (Adams et al., 2001), both interacting with specific tight (Wong et al., 1999) and adherens junctions (Tao et al., 1996) and intervening in focal adhesions via β1-integrin (Jawhari et al., 2003). Moreover, the protein promotes cell locomotion (Yamashiro et al., 1998) and participates in the mechanical organisation of stress fibres (Adams, 1997). A definite
overexpression of fascin has been reported in carcinomas of different organs, including oesophagus (Hashimoto et al, 2005a; Xie et al., 2005; Zhang et al., 2006), stomach (Hashimoto et al., 2004), colon (Jawhari et al., 2003; Hashimoto et al., 2006), pancreas (Maitra et al., 2002), breast (Grothey et al., 2000; Yoder et al., 2005), skin (Goncharuk et al., 2002), lung (Pelosi et al, 2003a,b; Choi et al., 2006), urinary bladder (Tong et al., 2005), and ovary (Hu et al., 2000) and the protein has been recently proposed as a novel biomarker for aggressive tumour behaviour (Hashimoto et al, 2005b, 2006; Zhang et al., 2006). In colonic adenocarcinoma cell lines, fascin overexpression correlates with an increase in the formation of dynamic cell protrusions, proliferation, and invasiveness (Jawhari et al., 2003). Besides, more recently, in CRC, fascin overexpression has been shown to correlate with proximal colon tumour location and shorter patients’ survival (Hashimoto et al., 2006).

In this study, we investigated the prevalence and clinicopathological implications, including survival, of fascin immunoreactivity in either primary tumours or lymph node metastases of 228 patients with stage III–IV colonic adenocarcinoma. Our results support the view that fascin correlates with a tumour cell invasive phenotype, emerging as an independent predictor of reduced survival.

MATERIALS AND METHODS

Patients and tumours

Three hundred consecutive patients with stage III and IV (6th TNM classification of malignant tumours) advanced colorectal adenocarcinoma (231 colonic and 69 rectal) were identified and surgically treated at the European Institute of Oncology and at the University Hospital of Verona between 1988 and 1999. As this study was aimed at assessing the role of fascin in tumour progression of advanced colonic adenocarcinoma, we also included stage IV patients in the survival analysis, whereas the earlier stages of disease were not taken into account. Other inclusion criteria were no neo-adjuvant treatment and age ≤ 85 years (three patients aged 87, 88, and 92 years were excluded). All the data were retrieved from the original clinical charts, databases of the participating institutions, through direct interviews with surviving patients or their family members, family physicians, or the death registry office. The data that was analysed included the date of birth, date of surgery, date of last follow-up/death, date of local recurrence, date of first metastatic spreading, site of metastatic spreading (classified as local recurrence, hepatic, abdominal extra-hepatic, and distant), and the anatomical location of the neoplasm (classified as right, transverse, and left colon).

Information on overall survival (OS) was available for all patients, with 141 of them (61.8%) dying from their disease. The mean survival period was 54.2 months (standard deviation 43.1; range 0–106 months). Recurrent disease information was assessable in 220 (96%) patients, with a mean time to event of 39.0 months (standard deviation 38.6; range 0–170 months). Information on disease-free survival was available for 205 patients (90%) (116 treated and 89 not treated). Because of the different biological, clinicopathologic, and theraputic implications of rectal cancer (with particular reference to the role of additional therapies in the management of these patients) (Rodel and Sauer, 2004), rectal adenocarcinomas were recorded for the prevalence of fascin immunoreactivity but then excluded from any further clinicopathologic and survival analysis.

The paraffin blocks of all cases were retrieved and the original haematoxylin and eosin-stained sections reviewed for confirmation of pT3 or pT4 status, histologic tumour grade (categorised with a three-tier grading system using the extent of gland formation and microtubular structures as the primary grade criteria (Grinnell, 1939; Jass et al., 1986), tumour type, occurrence of vascular (classified as intramural and extramural venous vessels or lymphatic channels), and perineural invasion; tumour growth patterns (expansive or infiltrative margins); number of metastatic lymph nodes, and the occurrence of extracapsular lymph node involvement.

Immunocytochemistry and evaluation of data

Formalin-fixed and paraffin-embedded tissue samples obtained at surgery were investigated. Tumours up to 2 cm in size were entirely embedded and immunostained. Ten samples of normal colonic wall from patients with nonmalignant intestinal diseases and peritumoural colonic tissue from the same cohort of patients investigated provided the control groups for noncarcinoma and carcinoma patients, respectively.

Fascin was immunolocalised on 4-μm-thin tissue sections using the monoclonal antibody 55k-2 (Dako, Glostrup, Denmark) recognising a 55-kDa protein in Western blots of HeLa, normal rat kidney, and gerbil fibroma cell lysates (Yamashiro-Matsumura and Matsumura, 1986). This anti-fascin antibody was also found to immunoblot bacterially expressed human homolog singed gene (hsn) product and a 55-kDa protein from cell lysates of peripheral blood dendritic cells (Duh et al., 1994; Mosialos et al., 1996). After blockage of endogenous peroxidase activity with 5% hydrogen peroxide and heat-mediated antigen retrieval with 1 mM EDTA, pH 8 (Sigma Chemical Co, St Louis, MO, USA) in a microwave oven at 750 W for 12 min, the sections were reacted overnight at 4°C with the primary antibody at a dilution of 2.5 μg ml⁻¹ in Tris-buffered saline. Detection was performed using a commercially available kit (Dako EnVision Plus-HRP, Dako), according to the manufacturer’s instructions. Peroxidase activity was developed with 3-3′-diaminobenzidine-copper sulphate (Sigma Chemical Co) to obtain a brownish-black end product. Tumour proliferative fraction was assessed by Ki-67 immunostaining and the percentage of reactive cells was recorded as the labelling index according to previously refined immunohistochemical method (Pelosi et al., 1996). The specificity of all immunoreactions was double-checked by substituting the primary antibodies with nonrelated isotypic mouse immunoglobulins at a comparable dilution and with normal mouse serum alone (Burry, 2000).

Two observers (GP and GP) evaluated fascin immunoreactivity of tumour cells independently and blindly, without knowledge of the patients’ clinical outcome or of the tumour features. Results were expressed according to the percentage of immunoreactive cells among at least 2000 tumour cells in consecutive unselected histological fields. Intensity of fascin immunostaining was also graded as low (expressed as ‘1’), if a faint to distinct granular staining was detectable throughout the cytoplasm but less intense than seen in the normal internal controls (endothelial and dendritic cells), or strong (expressed as ‘2’), if it was of the same or greater intensity than the control staining. A score was then calculated multiplying the percentage of immunoreactive cells by the intensity level of immunostaining. Therefore, tumours were considered negative if staining was completely absent in the neoplastic cells; moderately positive if the fascin score ranged from 1 to 100, and strongly positive if it was higher than 100.

Statistical analysis

Associations between intramural and extramural fascin expression and clinicopathological characteristics of the tumour were assessed by the Fisher’s exact test for categorical variables and with the Mantel–Haenszel χ² test for trend for ordinal variables. Disease-free survival was calculated from the date of surgery until the date of either loco-regional relapse or the development of distant metastasis. Only mortality from disease was considered for OS
analysis. Disease-free and OS curves were obtained using the Kaplan–Meier method, whereas the comparison between the groups was assessed by the log-rank test, considering a $P$-value $<0.05$ as significant. The univariate and multivariate Cox proportional hazard regression was used to assess the prognostic significance of fascin and other clinical characteristics on survival. As this study dealt with locally advanced and metastatic tumours that actually have a poor prognosis, patients were censored at 6 years to emphasize the prognostic role of fascin expression as an early predictive marker for tumour progression. All tests were two-sided. Analyses were performed with the SAS software (Cary, NC, USA).

RESULTS

Patients

The study encompassed 121 men (median age 66 years, range 38–84) and 107 women (median age 65, range 30–84).

Stage distribution included nine (4%) tumour patients with stage IIIA (six men and three women), 100 (44%) with stage IIIB (49 men and 51 women), 61 (26%) with stage IIIC (29 men and 32 women), and 58 (25%) with stage IV (37 men and 21 women). Eighty-five patients (37%) had right colon carcinoma, 14 (6%) transverse colon carcinoma, and 129 (57%) left colon carcinoma.

Figure 1 (A–D) Fascin expression in primary and metastatic lymph nodes, in conventional (A and B) and mucinous adenocarcinoma (C and D), both showing fascin score $>100$. Two other examples of adenocarcinoma (A) and lymph node metastasis (B) lacking any fascin immunoreactivity are also shown (insets).

Figure 2 The distribution of the fascin score along the large bowel according to the intramural (im), extramural (em), and lymph node (ln) compartments is shown. A differential distribution was noted among the diverse intestinal tracts ($P<0.001$), with a prevalence of positive fascin score in the intramural component of the right colon only as compared with the extramural or lymph node location ($P = 0.025$).
Table 1 Association between intramural and extramural fascin expression and other clinicopathological characteristics in colon cancer

|                      | Intramural fascin score | Extramural fascin score | Lymph nodal fascin score |
|----------------------|--------------------------|-------------------------|--------------------------|
|                      | Neg ≤ 100 > 100          | P-value                 | Neg ≤ 100 > 100          | P-value |
| Sex                  |                          |                         |                          |         |
| Male                 | 43                       | 54                      | 24                       |         |
| Female               | 23                       | 51                      | 33                       | 0.010   |
| Age                  |                          |                         |                          |         |
| <50                  | 10                       | 12                      | 3                        |         |
| 50–59                | 9                        | 29                      | 11                       |         |
| 60–69                | 23                       | 31                      | 13                       |         |
| 70+                  | 24                       | 33                      | 30                       | 0.120a  |
|                      |                          |                         |                          | 0.247a  |
|                      |                          |                         |                          | 0.489a  |
| Localisation         |                          |                         |                          |         |
| Right                | 6                        | 44                      | 35                       |         |
| Transverse           | 4                        | 4                       | 6                        |         |
| Left                 | 56                       | 57                      | 16                       | <0.0001 |
|                      |                          |                         |                          |         |
| Histology            |                          |                         |                          |         |
| Nonmucinous          | 65                       | 98                      | 50                       |         |
| Mucinous             | 1                        | 7                       | 7                        | 0.017   |
| Grade                |                          |                         |                          |         |
| G1                   | 5                        | 10                      | 1                        |         |
| G2                   | 51                       | 65                      | 28                       |         |
| G3                   | 10                       | 30                      | 28                       | <0.0001a|
|                      |                          |                         |                          |         |
| pT                   |                          |                         |                          |         |
| pT1                  | 0                        | 1                       | 1                        |         |
| pT2                  | 3                        | 8                       | 1                        |         |
| pT3                  | 56                       | 85                      | 44                       |         |
| pT4                  | 7                        | 11                      | 11                       | 0.38a   |
|                      |                          |                         |                          | 0.47a   |
|                      |                          |                         |                          | 0.042a  |
| pN                   |                          |                         |                          |         |
| N1                   | 42                       | 69                      | 28                       |         |
| N2                   | 24                       | 36                      | 29                       | 0.12    |
| Number of positive nodes |                      |                         |                          |         |
| 1                    | 18                       | 33                      | 11                       |         |
| 2                    | 19                       | 23                      | 10                       |         |
| 3–5                  | 23                       | 28                      | 18                       |         |
| >5                   | 6                        | 21                      | 18                       | 0.014a  |
|                      |                          |                         |                          | 0.0018a |
|                      |                          |                         |                          | 0.0079a |
| LN met               |                          |                         |                          |         |
| Intra LN             | 41                       | 65                      | 23                       |         |
| Extra LN             | 25                       | 40                      | 34                       | 0.019   |
|                      |                          |                         |                          | 0.0072  |
|                      |                          |                         |                          | 0.0081  |
| pM                   |                          |                         |                          |         |
| M0                   | 55                       | 80                      | 35                       |         |
| M1                   | 11                       | 25                      | 22                       | 0.0060  |
| Stage                |                          |                         |                          |         |
| IIIA                 | 3                        | 5                       | 1                        |         |
| IIIB                 | 30                       | 50                      | 20                       |         |
| IIIC                 | 22                       | 25                      | 14                       |         |
| IV                   | 11                       | 25                      | 22                       | 0.021a  |
|                      |                          |                         |                          | 0.051a  |
|                      |                          |                         |                          | 0.0064a |
| Ki-67 intramural     |                          |                         |                          |         |
| <40                  | 18                       | 26                      | 15                       |         |
| 40–59                | 21                       | 31                      | 12                       |         |
| 60+                  | 27                       | 46                      | 30                       | 0.40a   |
|                      |                          |                         |                          | 0.75a   |
|                      |                          |                         |                          | 0.89a   |
| Ki-67 extramural     |                          |                         |                          |         |
| <40                  | 26                       | 33                      | 16                       |         |
| 40–59                | 14                       | 21                      | 16                       |         |
| 60+                  | 23                       | 42                      | 24                       | 0.23a   |
|                      |                          |                         |                          | 0.89a   |
|                      |                          |                         |                          | 0.54a   |

LN, lymph node. No association was found between fascin expression and centre, chemotherapeutic treatment, vascular, lymphatic, and neural invasion. *p-value for trend using the Mantel–Haenszel $\chi^2$ test.
| Univariate analysis | Disease free survival | Overall survival (death from cancer) |
|---------------------|-----------------------|----------------------------------|
|                     | HR (95% CI)           | P-value                          | HR (95% CI)           | P-value |
| Fascin intramural   |                       |                                  |                       |
| Negative            | 1.00                  |                                  | 1.00                  |         |
| ≤ 100               | 1.38 (0.93–2.05)      | 0.11                             | 1.69 (1.11–2.59)      | 0.015   |
| > 100               | 1.64 (1.08–2.48)      | 0.020                            | 2.01 (1.25–3.23)      | 0.004   |
|                      |                       |                                  |                       |
| Fascin extramural   |                       |                                  |                       |
| Negative            | 1.00                  |                                  | 1.00                  |         |
| ≤ 100               | 1.31 (0.89–1.95)      | 0.15                             | 1.50 (1.00–2.26)      | 0.057   |
| > 100               | 1.53 (1.05–2.23)      | 0.028                            | 1.71 (1.13–2.60)      | 0.012   |
|                      |                       |                                  |                       |
| Fascin LN           |                       |                                  |                       |
| Negative            | 1.00                  |                                  | 1.00                  |         |
| ≤ 100               | 1.69 (1.16–2.45)      | 0.0058                           | 2.04 (1.37–3.05)      | 0.0005  |
| > 100               | 1.81 (1.20–2.71)      | 0.0045                           | 1.87 (1.20–2.90)      | 0.0056  |
|                      |                       |                                  |                       |
| Localization        |                       |                                  |                       |
| Left                | 1.00                  |                                  | 1.00                  |         |
| Right-transverse    | 1.42 (1.04–1.95)      | 0.029                            | 1.44 (1.03–2.01)      | 0.033   |
|                      |                       |                                  |                       |
| Histology           |                       |                                  |                       |
| Nonmucinous         | 1.00                  |                                  | 1.00                  |         |
| Mucinous            | 1.66 (0.94–2.94)      | 0.083                            | 2.11 (1.19–3.75)      | 0.011   |
| Grade               |                       |                                  |                       |
| G1                  | 1.00                  |                                  | 1.00                  |         |
| G2                  | 2.05 (0.96–4.42)      | 0.083                            | 2.44 (0.97–6.01)      | 0.052   |
| G3                  | 2.43 (1.10–5.37)      | 0.029                            | 2.96 (1.17–7.49)      | 0.022   |
| PT                  |                       |                                  |                       |
| pT1–2               | 1.00                  |                                  | 1.00                  |         |
| pT3–4               | 4.01 (1.48–10.8)      | 0.0062                           | 2.93 (1.08–7.92)      | 0.034   |
| PN                  |                       |                                  |                       |
| N1                  | 1.00                  |                                  | 1.00                  |         |
| N2                  | 1.74 (1.27–2.39)      | 0.0006                           | 1.56 (1.11–2.19)      | 0.0096  |
| Number of positive nodes |                 |                                  |                       |
| 1                   | 1.00                  |                                  | 1.00                  |         |
| 2–5                 | 1.65 (1.10–2.45)      | 0.015                            | 1.59 (1.03–2.46)      | 0.035   |
| > 5                 | 2.91 (1.82–4.63)      | < 0.00001                        | 3.30 (2.03–5.37)      | < 0.0001|
| LN met              |                       |                                  |                       |
| Intra LN            | 1.00                  |                                  | 1.00                  |         |
| Extra LN            | 1.79 (1.31–2.45)      | 0.0003                           | 1.79 (1.28–2.50)      | 0.0007  |
| PM                  |                       |                                  |                       |
| M0                  | 1.00                  |                                  | 1.00                  |         |
| M1                  | 3.67 (2.62–5.15)      | < 0.0001                         | 5.93 (4.13–8.52)      | < 0.0001|
| Stage               |                       |                                  |                       |
| IIIA                | 0.30 (0.07–1.12)      | 0.091                            | 0.43 (0.11–1.77)      | 0.24    |
| IIIB                | 1.00                  |                                  | 1.00                  |         |
| IIIC                | 1.75 (1.17–2.63)      | 0.0065                           | 1.62 (1.04–2.52)      | 0.033   |
| IV                  | 4.31 (2.94–6.32)      | < 0.0001                         | 6.92 (4.56–10.5)      | < 0.0001|
| Venous invasion     |                       |                                  |                       |
| Absent              | 1.00                  |                                  | 1.00                  |         |
| Present             | 1.64 (1.20–2.25)      | 0.0020                           | 1.65 (1.18–2.31)      | 0.0035  |
Immunohistochemical data

The normal colonic epithelium consistently lacked any fascin immunoreactivity, which was instead encountered in the underlying lamina propria and tumour stroma, with the endothelial and fibroblastic cells showing a consistent and strong immunoreactivity for fascin.

Representative examples of fascin immunoreactivity are depicted in Figure 1, and its distribution along the large bowel according to intramural, extramural, and lymph node location is reported in Figure 2. A positive fascin score was detected in the intramural component of 162 (71%) specimens (intramural fascin), in the extramural component of 132 (58%) (extramural fascin), and in metastatic lymph nodes of 141 (62%) patients.

Thirty-five tumours showed a positive fascin score in the intramural but not in the extramural component; the opposite was seen in only four tumours.

Among the 162 tumours positives in the intramural component, 130 had positive lymph node metastasis (80%) and among the 132 tumours positives in the extramural component 116 had positive lymph node metastasis (88%).

Striking differences in the prevalence of fascin immunoreactivity between right- and left-sided colon segments were found (Figure 2 and Table 1).

An intratumoural, extratumoural, and lymph node fascin score >100 was significantly associated with the female sex, tumour location in the right or transverse colon, more advanced tumour stage, pN class, the pattern of lymph node involvement (with a higher level of immunoreactivity in lymph nodes showing extracapsular extension), higher tumour grade, mucinous histotype, and the occurrence of metastasis and of a heavy peritumoural lymphoid infiltrate (Table 1). No correlations were found with the other variables, including tumour proliferative fraction, chemotherapeutic regimen (data not shown), and site of metastasis.

Regarding survival, traditional prognostic variables such as the pT, pN, tumour grade, and vascular invasion correlated well with the patients’ life expectation (Table 2), with tumour stage according to the TNM system being a strong predictor of survival (Figure 3). A correlation with poorer clinical outcome was also noted for mucinous differentiation and right tumour location (Table 2).

A positive fascin score (independent of its threshold) at any site was significantly associated with both a shorter OS and DFS, together with stage and a mucinous-signet ring histotype (Table 3).
DISCUSSION

In this study, we investigated a large series of colorectal carcinomas for fascin immunoreactivity. The main results are that fascin is upregulated in most colonic carcinomas, correlating with a higher tumour grade, right tumour location and tumour stage, and that fascin immunoreactivity is an independent predictor of reduced OS and DFS in patients with advanced tumour stage.

A growing body of literature reports that fascin is expressed in many types of transformed epithelial cell lines and in several solid neoplasms (Grothey et al, 2000; Hu et al, 2006; Goncharuk et al, 2002; Jawhari et al, 2003; Pelosi et al, 2003a, b; Hashimoto et al, 2004, 2005a, 2006; Tong et al, 2005; Xie et al, 2005; Yoder et al, 2005; Choi et al, 2006; Zhang et al, 2006) where it correlates with tumour stage (Hashimoto et al, 2004, 2005a; Yoder et al, 2005; Choi et al, 2006) and grade (Pelosi et al, 2003b; Hashimoto et al, 2004, 2005a; Yoder et al, 2005), pT class (Hashimoto et al, 2004, 2005a), lymph node involvement (Hashimoto et al, 2004, 2005a; Choi et al, 2006; Zhang et al, 2006), recurrence (Hashimoto et al, 2004), and both OS (Hashimoto et al, 2004, 2005a, 2006; Yoder et al, 2005) and DFS (Pelosi et al, 2003b; Yoder et al, 2005). The close association we found between fascin immunoreactivity and tumour stage, tumour grade, the number and type of lymph node involvement, and distant metastasis indicates the major role of fascin in the progression of colonic adenocarcinoma. In our study, fascin seems to play a different role in the progression of right- and tranverse-sided neoplasms (as recently evidenced) (Hashimoto et al, 2006) and of mucinous variants of colonic

![Graphs showing disease-free and overall survival analysis](image)

Figure 4  (A) Disease-free and OS analysis of 228 colon adenocarcinoma patients according to intramural, extramural, and lymph node fascin expression. (B) Overall survival of patients with stage III (top) and IV (bottom) colon adenocarcinoma according to intramural, extramural, and lymph node fascin expression.
adenocarcinomas as compared with the other types of colonic cancers (Table 2). Pelosi et al (2003b) also noted a differential distribution of fascin immunoreactivity in mucinous and non-mucinous bronchioloalveolar carcinomas of the lung and fascin transfection in colonic carcinoma cell lines increased the levels of neutral mucin (Jawhari et al, 2003). Although the clinical implications of colorectal mucinous carcinoma are still controversial, a consensus conference on colorectal carcinomas stated that mucinous and signet ring carcinomas, when evaluated jointly, have a poorer prognosis than intestinal-type adenocarcinomas (Compton et al, 2000).

Although the association of fascin expression with lymph node involvement has already been documented for other gastrointestinal cancers such as oesophageal and gastric carcinomas (Hashimoto et al, 2004, 2005a) and also for pulmonary neoplasms (Pelosi et al, 2003a; Choi et al, 2004), we have shown that fascin immunoreactivity in colonic adenocarcinomas is also related to the patterns of lymph node involvement (intra- vs extra-lymph nodal colonisation). As fascin immunoreactivity is also associated with the number of metastatic lymph nodes and the occurrence of distant metastases, we hypothesise that this molecule is very likely involved in the metastatic process of colonic adenocarcinoma cells via its motility-inducing properties. This is further sustained by the observation that patients with lymph node metastases immunoreactive for fascin experienced a more aggressive clinical course than patients with negative lymph node metastases (Figure 4A). This significance was kept stratifying the prognostic impact of fascin according to tumour stages III and IV (Figure 4B).

Thus, fascin expression in primary and metastatic tumours could unveil the different clinical aggressiveness of tumours that are otherwise classified in the same risk category.

Fascin immunoreactivity was not associated with the tumour proliferation fraction as assessed by the Ki-67-labelling index, at variance with previous studies of lung and stomach cancers and also of colonic cell cultures (Jawhari et al, 2003; Pelosi et al, 2003b; Hashimoto et al, 2004). In a recent work on CRC, similar to the current one, comparing fascin expression with Ki-67 immunostaining, a lack of direct association between the two markers was noted, indicating that the fascin upregulation do not correlate positively with cell proliferation (Hashimoto et al, 2006).

However, the prognostic role of the Ki-67-labelling index in colorectal carcinoma is still highly controversial and probably different from other solid neoplasms. In fact, recent observations document that a high Ki-67-labelling index is associated with better OS (Allegra et al, 2003) in both treated and untreated patients (Garrity et al, 2004), as reported in the current series (Table 2).

Fascin immunoreactivity was associated with a shorter OS and DFS, independent of tumour stage, which is the most important prognostic factor in this tumour type (Compton and Greene, 2004). In the multivariate analysis, similar results were also obtained for other tumour types, such as pulmonary (Pelosi et al, 2003a), oesophageal (Hashimoto et al, 2005a), breast (Yoder et al, 2005) carcinomas and more recently CRC (Hashimoto et al, 2006). Our findings confirming that fascin is a negative prognostic factor for advanced colonic adenocarcinoma encourage clinical translation, especially when considering that the current substaging of colorectal cancer according to the latest TNM classification emphasizes the prognostic heterogeneity of patients within the same tumour stage group. The different prognostic implications of lymph node metastases according to the amount of fascin could well be incorporated in new staging proposals. Finally, the identification of patients with a reduced life expectation according to the degree of fascin expressed by their respective tumours also justifies the potential use of novel targeted therapies, as recently proposed for other malignant epithelial neoplasms (Hashimoto et al, 2005b, 2006).

Additional studies are needed to investigate the role of fascin in right-sided colonic cancer and in mucinous differentiation.

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Table 3  Multivariate analysis for disease-free and overall survival

| Disease free survival | Fascin | Histology | Stage | LN | At any site |
|-----------------------|--------|-----------|-------|----|------------|
| Intramural            | 1.52 (1.04–2.23) | 1.38 (0.77–2.47) | 0.97 (0.97–1.07) | 1.94 (1.29–2.93) | 4.63 (3.09–6.92) |
| Extramural            | 1.54 (1.09–2.19) | 1.32 (0.73–2.37) | 0.72 (0.95–1.00) | 1.84 (1.22–2.76) | 4.78 (3.20–7.14) |
| LN                    | 1.72 (1.22–2.45) | 1.27 (0.70–2.28) | 0.39 (0.07–1.19) | 1.78 (1.18–2.67) | 4.51 (3.02–6.74) |
| At any site           | 1.61 (1.07–2.42) | 1.39 (0.78–2.50) | 0.34 (0.08–1.41) | 1.95 (1.29–2.94) | 4.74 (3.17–7.08) |

LN, lymph node. Also adjusted for age and sex. *Only variables that retains statistical significance were kept in the final model. Bold numbers indicate a P-value <0.05.

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