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Original contribution

Intense cytoplasmic ezrin immunoreactivity predicts poor survival in colorectal cancer☆

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Summary Ezrin is a membrane-cytoskeleton anchor, which, in experimental models, regulates tumor cell invasion and metastatic ability. We carried out immunohistochemical analysis of ezrin in 74 advanced colorectal cancer patients and correlated it to clinicopathologic variables and disease outcome. In contrast to the predominantly membraneous immunoreactivity of normal colorectal epithelium, ezrin expression in the colorectal cells was typically cytoplasmic. Altogether, 16.2% (12/74) of the tumors showed negative/weak ezrin staining, 35.1% (26/74) had moderate staining, and 48.6% (36/74) had intense staining. The expression was more intense in colon than in rectal carcinomas (P = .003). Increased ezrin expression was associated with adverse outcome, that is, shorter disease-specific survival; 48.3 months and 36.6 months for negative-weak versus intense expression (P = .041) as well as shorter survival with metastases at 36 months (P = .030); the metastases_{36} rates in $\text{ezrin}_{\text{neg/weak}}$, $\text{ezrin}_{\text{moderate}}$, $\text{ezrin}_{\text{intense}}$ are 58.3%, 25.0%, and 18.4%, respectively. In univariate survival analysis, dichotomized (negative/weak versus moderate/strong) ezrin expression significantly predicted both the 5-year disease specific survival (P = .035) and 5-year metastases (P = .018) but lost this predictive power in multivariate (Cox) analysis. High ezrin expression was also related to high E-cadherin (cytoplasmic) expression, DNA aneuploidy, and high thymidylate synthase expression (P = .046, P = .042, P = .046, respectively). These results suggest that ezrin may play a role in colorectal cancer progression and that ezrin expression might provide clinically valuable information in predicting the biological behavior of colorectal cancer.

1. Introduction

The prognosis of colorectal cancer (CRC) patients depends on the development of recurrence and/or metastasis [1]. However, one of the major problems is the definition of reliable criteria for predicting recurrence and for identifying the tumors that will respond to chemotherapy [1]. The

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metastatic cascade starts with a breakdown of the epithelial integrity, which enables tumor cells to invade the surrounding stroma, penetrate either into blood or lymphatic vessels, and finally infiltrate into the appropriate target organs. Among others, cell adhesion molecules and actin cytoskeleton play a role in these processes [2-4].

Ezrin is a member of the ezrin-radixin-moesin (ERM) family of proteins that link the actin-containing cytoskeleton to the plasma membrane molecules and play a role in the control of actin cytoskeleton [5]. Ezrin also participates in cell signaling involved in the regulation of cell survival, proliferation, and migration [6]. The functions of ezrin may be mediated by its direct or indirect interactions with adhesion molecules such as E-cadherin/catenin complex. However, ezrin is also essential for the maintenance of cell-cell adhesion and is inhibitory toward cell-matrix adhesion in human colonic epithelial cells [2].

Several recent studies have addressed the role of ezrin in the behavior and outcome of human malignancies [4,7]. These data suggest that up-regulation of ezrin correlates to invasive characteristics of malignant cell lines [8,9] as well as to aggressiveness, metastasic spread, and poor clinical outcome of various human cancers [10-16]. Furthermore, in vitro and in vivo studies implicate ezrin as an important molecule in invasive/metastatic processes [17-22]. Until now, there have been no data on the expression of ezrin in CRC.

In the present study, we quantified the expression of ezrin in a cohort of 74 CRC patients, with special reference to its associations with key clinicopathologic features as well as its value as a predictor of treatment response and disease outcome.

Table 1 Key characteristics of the patients and their tumors

Variable	n or value	% [a]
Patients	74	
Male	46	62.2
Female	28	37.8
Age (y)		
Median (range)	61.5 (24-78)	
Primary tumor status [b]		
T1	1	1.4
T2	5	6.8
T3	52	70.2
T4	13	17.6
Tx	3	4.0
Primary nodal status [b]		
N0	20	27.0
N+	42	56.8
Nx	12	16.2
Histologic grade		
I	11	14.9
II	50	67.6
III	12	16.2
x	1	1.4
Stage		
II	13	17.6
III	17	23.0
IV	44	59.4
Metastases at diagnosis [b]		
M0	30	40.5
M1	44	59.5
Survival (m)		
From primary diagnosis (median [range])	30.8 (5-150)	
From metastasis (median [range])	22 (3-75)	

x indicates status not known.
[a] When applicable.
[b] TNM classification.

2. Materials and methods

2.1. Study material

The material of the present study consists of a series of 74 patients with advanced CRC, of whom 44 had metastases at diagnosis (stage IV disease), whereas the remaining developed metastatic disease within a median of 17.1 months (range, 2.8-141.7 months). The patients were treated at the Department of Oncology and Radiotherapy, Turku University Hospital (Turku, Finland), and enrolled into a retrospective study between October 1998 and August 2003. The patients were followed-up until March 2007, for a median of 30.8 months (range, 4.7-149.8 months). The study was approved by the Turku University Hospital Ethics Committee and was conducted in accordance with the Declaration of Helsinki. Samples were collected with the endorsement of the Finnish National Authority for Medicolegal Affairs.

The key clinical data of the patients are shown in Table 1. Of these 74 cases, 28 were women and 46 were men. The mean age was 61.5 years (range, 24-78 years). The majority (n = 28) of the tumors were localized in the descending colon, followed in the order of frequency by the ascending colon (n = 26), rectum (n = 14), and colon transversum (n = 6). At the time of diagnosis, 13 patients were stage II, 17 stage III, and 44 patients were stage IV. Accordingly, most (n = 52, 70.3%) had T3 tumors and had lymph node involvement at the time of diagnosis (n = 54). The patients were selected in the cohort based on both the diagnosis and the treatment they received and assigned to 1 of the 2 treatment arms: (i) 16 were treated with irinotecan alone, and (ii) 58 received a combination of irinotecan and 5-fluorouracil (5-FU).

2.2. Ezrin immunohistochemistry

Sections were deparaffinized in xylene and rehydrated through the graded series of ethanol, followed by rinsing in Tris-buffered saline. Immunohistochemical staining was performed with a Techmate 500+ immunostaining device using a peroxidase/diaminobenzidine multilink detection kit (Dako, Glostrup, Denmark) that is based on an indirect streptavidin-biotin method. The primary antibody (murine monoclonal IgG antibody to human ezrin, clone 3C12,