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ORIGINAL RESEARCH

LFA-1 Expression in a Series of Colorectal Adenocarcinomas

Maria G. Papas · Pantelis S. Karatzas · Ioannis S. Papanikolaou · Evanthia Karamitopoulou · Eumorphia M. Delicha · Andreas Adler · Konstantinos Triantafyllou · Georgia-Heleni Thomopoulou · Efstratios Patsouris · Andreas C. Lazaris

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Abstract
Introduction LFA-1 is an adhesion molecule which belongs to the β2-integrin family. Overexpression of LFA-1 in hepatic natural killer cells has been associated with increased apoptosis of neoplastic cells in colorectal cancer (CRC); moreover, studies in CRC have linked LFA-1 overexpression in neoplastic cells with vascular intrusion through adhesion to endothelial cells, thus implying a possible role in creation of metastases.
Aims and Methods We studied the expression of LFA-1 in a series of 82 patients with CRC. A standard three-step immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded tissue sections. An IgG2a anti-CD11a monoclonal antibody was used. Cases were characterized according to clinicopathological variables including sex, age, tumor localization, size, grade, Dukes stage, wall invasion, and presence of metastatic lymph nodes (mLNs) or distal metastases.
Results LFA-1 was expressed at the primary tumor site in 51 cases and 6/33 cases with metastatic lymphnodes. In Dukes D cases (n=4), only one case was LFA-1(+). LFA-1 expression at the primary tumor site was associated with the absence of metastatic disease and with Dukes B stage. However, in those cases with LFA-1 expression in cancer cells in mLNs, this was associated with its expression at the primary tumor site.
Conclusion The positive association of LFA-1 expression in mLNs when the primary tumor site is also LFA-1(+) could imply an adaptation advantage of this specific cellular clone to its micro-environment, predisposing it to creation of mLNs, pointing to a role for LFA-1 in creation of mLNs in CRC.

Keywords Integrins · LFA-1 · Metastases · Colorectal cancer

M. G. Papas · E. Karamitopoulou · G.-H. Thomopoulou · E. Patsouris · A. C. Lazaris
First Department of Pathology, Faculty of Medicine, University of Athens, Athens, Greece

P. S. Karatzas · I. S. Papanikolaou · E. M. Delicha · K. Triantafyllou
Hepatogastroenterology Unit, 2nd Department of Internal Medicine-Propaedeutic, Attikon University General Hospital, University of Athens, Athens, Greece

I. S. Papanikolaou · E. M. Delicha · A. Adler
Central Interdisciplinary Endoscopy Unit, Charite University Hospitals, Campus Virchow Hospital, Berlin, Germany

I. S. Papanikolaou (✉)
Central Interdisciplinary Endoscopy Unit, Department of Gastroenterology, Hepatology and Metabolic Diseases, Charité Medical University of Berlin, Campus Virchow Clinic, Augustenburger Platz 1, 13353 Berlin, Germany
e-mail: ispapn@hotmail.com

Introduction

Colorectal cancer (CRC) has a high rate of morbidity and mortality and constitutes one of the greatest hazards for public health, especially in the industrialized world. Every year, about 945,000 people develop CRC and around 492,000 die [1]. This high annual mortality highlights the need for a better understanding of the various steps in the pathogenesis of CRC, which might lead to new therapeutic strategies [2]. Migration of neoplastic cells is a vital step in the process of human carcinogenesis as it constitutes the

pathophysiological basis of metastasis. This procedure includes adhesion of malignant cells to the endothelium of vessels in order to invade into the surrounding tissue. Adhesion molecules play a major role in this process, which is similar to that used by lymphocytes to migrate into tissue during inflammation. This includes the interaction of lymphocytes with the endothelial cells of vessels and particularly with cells that express adhesion molecules [3, 4]. Integrins comprise a well-studied group of adhesion molecules that play a pivotal role in this process. They are transmembrane glucoproteins, consisting of α and β chain, with tight conjunctions to each other. There are three subgroups of integrins presently known, according to the type of β chain, namely β1, β2, and β3. LFA-1 (CD11a/CD18) is an adhesion molecule which belongs to the β2-integrin family; there seems to be data linking its overexpression in neoplastic cells from CRC with vascular intrusion through adhesion to endothelial cells, implying a possible role for this molecule in the creation of metastases [5, 6].

In the present study, we analyzed the expression of LFA-1 in a series of CRC histological specimens and attempted to investigate its possible role in the creation of metastasis.

Methods

Our series included histological specimens from 82 consecutive patients who underwent colectomy in a 12-month period due to a diagnosis of colorectal adenocarcinoma of conventional histologic type; all cases were examined in the 1st Department of Pathology of the University of Athens and were characterized according to the following clinicopathological variables: sex (male/female, 52/30), age (mean, 74.5), tumor localization (rectum, 27; sigmoid, 26; descending colon, 4; transversum, 5; ascending colon, 10; cecum, 10; i.e., 34%, 33%, 5%, 4%, 12%, 12%), size (mean, 4.4 cm; median, 4 cm; range, 1.2–10 cm), grade of differentiation (24 highly differentiated, 48 medium, 10 low grade, i.e. 29.3%, 58.5%, and 12,2% respectively), Dukes stage (A, 5; B, 44; C, 29; D, 4; i.e. 6.1%, 53.7%, 35.4%, and 4.9% respectively), wall invasion and presence of metastatic lymph nodes (mLNs) or distal metastases (33 cases with versus 49 cases without infiltrated lymph nodes; 40.25% and 59.75%, respectively). In order to improve the reliability of statistical analysis, malignant adenomas were categorized in two major groups, i.e., left colon and rectum: 57 cases (69.5%) and right colon: 25 cases (30.5%).

Immunochistochemistry—Image Analysis

Expression of LFA-1 (CD11a) was examined as follows: a standard 3-step immunohistochemical analysis (ABComplex) was performed on formalin-fixed, paraffin-embedded tissue sections. An IgG2a anti-CD11a monoclonal antibody (Ancell, Bayport, MN, USA) was used. Exposure of antigenic epitopes was conducted in a microwave oven. Tissue sections of hypertrophic tonsils served as positive markers. Tissue sections which were not incubated with the examined antibody served as negative controls. Images were acquired using a Zeiss Axiolab microscope (Carl Zeiss GmbH, Jena, Germany) with a mechanical stage, fitted with a Sony CCD video camera (Sony, Tokyo, Japan). The video camera was connected in a Pentium II PC, loaded with the appropriate image analysis software (Sigma Scan Pro; Science, Erkrath, Germany). Slides were examined at ×200 magnification. The ratio expressed in percentile proportion (%) between the number of immunohistochemically positive-stained neoplastic cells and the total number (stained and unstained) was calculated, i.e., stain intensity was expressed as the number of immunopositive neoplastic cells divided by the total cell number (expressed in %).

Statistics

For the needs of statistical analysis, CD11a cases were categorized as positive and negative according to stain intensity (0–1% was considered negative/minimal expression, whereas ≥2% was considered positive). Chi-square test was used for the correlation between CD11a and the following parameters: age, tumor localization, grade of differentiation, transmural expansion, mLNs existence, and Dukes' stage. As for the correlation between CD11a and age or tumor size, Mann–Whitney non-parametric test was used.

Results

CD11a was detected mainly on the malignant cells' membranes (Figs. 1 and 2) in 51 cases of primary tumors (62% of all cases). In the cases with metastatic CRC (Fig. 3), it was detected at the primary site level in only 6/33 cases (18%). On the other hand, in the four cases of Dukes stage D CRC, the marker was detected in only one metastatic focus in the liver without being expressed in the primary site. The intensity of staining was not included in our statistical analysis but correlated to the immunopositivity of cases, reaching 96% and 92% of malignant cells in the primary tumor and in the metastatic foci of positive cases, respectively. As mentioned above, in order to define the malignant cells' immunopositivity for CD11a, specimens were divided in two groups: 0-1% was considered negative/minimal expression, whereas ≥2% was considered positive. According to these definitions, expression of CD11a was considered negative/minimal at the primary tumor site in 35 of the total 82 cases, whereas in the other

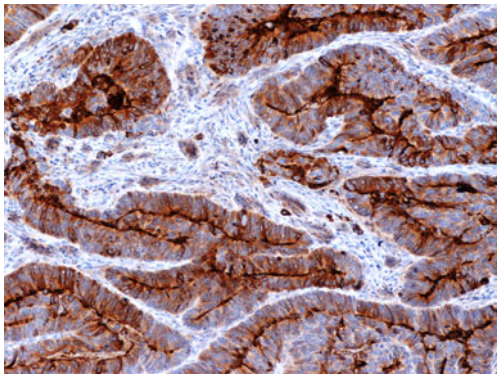

Fig. 1 Membranous LFA-1 immunoreactivity at the primary site of a low-grade colon adenocarcinoma (immunoperoxidase stain, ×100)

47 cases it was considered positive (i.e., 42.7% and 57.3%, respectively). Similarly, in the 33 metastatic cases, expression of CD11a in mLNs was considered negative/minimal in 27 and positive in 6 cases (81.8% and 18.2% respectively). CD11a expression at the primary tumor site was not associated to sex (p=0.747), tumor localization (p=0.248), differentiation (p=0.929), or transmural expansion (p=0.999). Moreover, there was no association between CD11a expression at the primary tumor site and age or tumor size (p=0.461 and p=0.763, respectively). In contrary, CD11a expression was related to the absence of metastases (p=0.044) and Duke's stage (B or C) (p=0.026).

CD11a expression in mLNs was not found to be associated to sex (p=0.062), localization (p=0.3), differentiation (p=0.666), transmural expansion (p=0.999), or Dukes' stage (C or D) (p=0.999) nor was there an association to age or tumor size (p=0.678 and p=0.212, respectively). However, an association was found between the molecule's expression in mLNs and its expression in the primary tumor site, i.e., in cases with infiltrated lymph nodes, expression of CD11a at the primary tumor site was related to a greater possibility of expression in the infiltrated nodes (p=0.002, the non-linear factor of Spearman r was 0.523).

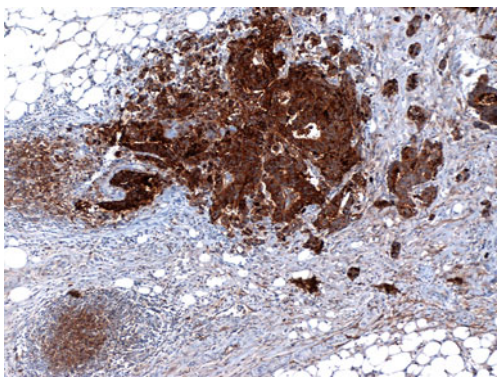

Fig. 2 Intense LFA-1 immunostaining at the invasive tumor margin of a colon adenocarcinoma. Some staining is noticeable in the lymphoid follicles surrounding the tumor cells (immunoperoxidase stain, ×50)

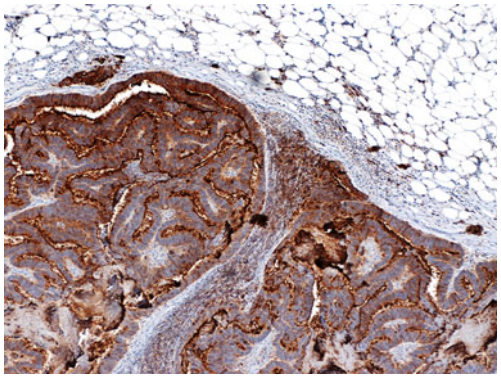

Fig. 3 LFA-1 immunodetection in cancer cells from a metastatically infiltrated regional lymph node from the case in Fig. 1 (immunoperoxidase stain, ×50)

Discussion

In our study, expression of CD-11a (which is a distinct chain of LFA-1, quite specific of its presence) was used as a marker of LFA-1. CD-11a showed high rates of immunopositivity in malignant epithelial cells, contrary to its low expression in lymphocytes of the lamina propria. These rates even reached—in some cases—96% of the malignant cells in the examined specimens from the primary tumor site and 92% for malignant cells from mLNs. The exact role of these adhesion molecules in the process of carcinogenesis and metastasis largely remains unclear. Presence of LFA-1—using a different methodology than the one used in this study—was examined in a small CRC series and its expression was found to be reduced in the lymphocytes of these tumors [7]. On the other hand, it has also been shown that LFA-1 overexpression in hepatic natural killer cells was associated with increased apoptosis of CRC neoplastic cells, which could imply a possible role for the molecule in colorectal carcinogenesis [8]. Lack of concrete data has left the question of such an association as well as the exact role of this molecule and its expression widely unresolved.

Cell migration involves similar mechanisms, irrespective of the background where it takes place, including inflammation, hematological malignancies, or solid malignancies. The main similarity is that the migrating cells—lymphocytes in inflammation and neoplastic cells in malignancies—adhere to the endothelial cells of the vessels via specific adhesion molecules expressed on both kinds of cells. It seems that these molecules, especially integrins, which under normal, low-stress conditions remain unexpressed can be activated under high-stress conditions (including neoplasia) via a number of factors (e.g., cytokines). This activation results in changes of the cytoskeleton as a result of F-actin polymerization which, in turn, can play a role in the metastatic process [9–14]. Fujisaki et al. examined the

role of LFA-1 in cases of carcinoma of the large intestine and the relation of LFA-1 expression to inflammatory stimulants [15]. It has been shown that presence of the receptor of hyalouronic acid (CD44) or of degraded hyalouronic acid might play the role of such a stimulant for the expression and activation of LFA-1. Moreover, these stimulants can induce the expression of the hepatocyte growth factor, which has been shown to increase the adhesion of malignant cells via LFA-1. Therefore, CD-44, a stimulant of LFA-1 activation, can participate in a direct and indirect signal transmission procedure, which induces the adhesion of malignant cells to the endothelium, assisting migration to the surrounding tissue [16, 17].

In this analysis for LFA-1, in regards to its relation to metastasis, our results showed that non-metastatic CRC was more likely to express LFA-1 compared to cases with infiltrated lymph nodes (and Dukes stage B had a higher positivity rate to the molecule than stage C). This could imply that LFA-1 has a negative relation to the metastatic procedure; however, on the other hand, LFA-1 expression in mLNs was found to be highly associated with its expression in the primary tumor site. An explanation for this could be that, in metastatic tumors with high positivity for LFA-1 expression, the molecule could participate in the migration process, whereas in metastatic CRC with no LFA-1 expression other factors (e.g., E-cadherins or catenins) might play such a role instead. Therefore, these results indicate that, although LFA-1 has a positive relation to a non-metastatic disease, it could still contribute to the whole metastatic process and migration of malignant cells. For example, the positive association of LFA-1 expression in mLNs with LFA-1 positivity at the primary tumor site as well as the fact that the intensity of staining correlated to immunopositivity of cases, i.e., when a case was positive, there was a big chance that it would display highly intense staining (in other words, a high rate of positive cells), could imply an adaptation advantage of this specific cellular clone to its micro-environment, predisposing it to creation of mLNs. Some of the latest advances in modern oncology are consistent with this possibility as the development of antitumor drugs aiming against adhesion molecules, including intergrins, has shown promising results [18]. Clearly, there are limitations to our study: Although we performed a quantification of immunohistochemichal signals, it would be interesting to confirm our results with a "pure" quantitative method (e.g., Western blot). Moreover, we acknowledge the fact that the cutoff level of 0–1% and ≥2 for negative/minimal expression and immunopositivity was set somewhat arbitrarily, but we feel that it is in accordance to what a pathologist would consider to be the cutoff level between "negative" and "positive" cases in a qualitative examination and therefore use it as a "standard" cutoff level [19]. Finally, the number of patients with CRC in our series is somewhat small and could be further increased (possibly to include more rectal carcinomas and Dukes stage D patients), but these issues exceeded the scope and the capabilities of the current study and could be addressed in future research; the aim of this study was merely to investigate the role of LFA-1 in CRC metastasis which, despite these shortcomings, seems to have been done.

In conclusion, our data imply that LFA-1 might be involved in the metastatic procedure in CRC. The exact mechanisms that are implicated remain obscure, which warrants conduction of further studies to clarify its involvement in the pathogenesis of metastasis in CRC and its role in colorectal carcinogenesis.

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