Annals of *Research* in *Oncology*
Vol. 3(4), 155-164, 2023

RESEARCH ARTICLE

L1CAM EXPRESSION IN THE PERITUMORAL STROMAL CELLS OF THE MICROENVIRONMENT OF COLORECTAL CANCER: A NEW TARGET FOR ONCOLOGISTS?

Flaviana **Cau** [1,*], Clara **Gerosa** [1,*], Pina **Ziranu** [2], Andrea **Pretta** [2], Monica **Piras** [1], Matteo **Fraschini** [3], Peter **Van Eyken** [4], Giorgio **La Nasa** [5], Massimo **Castagnola** [6], Ferdinando **Coghe** [7], Germano **Orrù** [8], Luigi **Zorcolo** [9], Andrei **Turtoi** [10], Antonella **Balestrieri** [11], Luca **Saba** [11], Mario **Scartozzi** [2,†], Gavino **Faa** [1,12,†]

[1] Division of Pathology, Department of Medical Sciences and Public Health, AOU of Cagliari, University of Cagliari, Cagliari, Italy
[2] Department of Medical Sciences and Public Health, Unit of Medical Oncology, University of Cagliari, Cagliari, Italy
[3] Department of Electrical and Electronic Engineering, University of Cagliari, Cagliari, Italy
[4] Department of Pathology, Genk Regional Ziekenhuis, 3600 Genk, Belgium- B-3200
[5] Department of Medical Sciences and Public Health, Hematology Unit, University of Cagliari Cagliari, Italy
[6] Proteomics Laboratory, Centro Europeo di Ricerca sul cervello, IRCCS Fondazione Santa Lucia, Rome, Italy
[7] Department of Services, Unit of Clinical Laboratory, AOU of Cagliari, Cagliari, Italy
[8] Molecular Biology Service Laboratory, Department of Surgical Science, University of Cagliari, Cagliari, Italy
[9] Department of Surgical Sciences, Unit of Colorectal Surgery, AOU of Cagliari, University of Cagliari, Cagliari, Italy
[10] Institut de Recherche en Cancérologie de Montpellier, INSERM U1194, Université Montpellier, Montpellier, France
[11] Department of Medical Sciences and Public Health, Unit of Radiology, University of Cagliari, Cagliari, Italy
[12] Department of Biology, College of Science and Technology, Temple University, Philadelphia, PA, USA
[†] Equal contribution
* Correspondence to: ✉ flacau@tiscali.it, https://orcid.org/0000-0001-6205-4555. ✉ clarge@tiscali.it, https://orcid.org/0000-0003-2561-5055.

ABSTRACT: According to the seed and soil theory, also known as the Paget's hypothesis, the tumor microenvironment (TME) plays a key role in promoting the onset and progression of cancer. In particular, fibroblasts and macrophages of the TME have been shown to regulate virtually all key tumor characteristics, from invasion to metastasis and resistance to therapy. There are numerous molecules that confer protumorigenic properties to the cells of the TME. The aim of this study was to analyze in a series of colorectal cancers the expression of the L1 cell adhesion molecule (L1CAM) in the cells of the TME, in order to better clarify the role of these cells in colorectal cancer (CRC) onset and progression. In the present study, 48 patients with CRC, aged between 38 and 88 years, were analyzed. L1CAM expression in the cells of the TME was detected in 35/48 cases (73%). L1CAM expression did not always parallel immunostaining for the adhesion molecule in tumor cells. Four groups were identified: group 1, stromal cells+/Tumor- (15/48 cases); group 2, stromal cells-/Tumor+ (19/48 cases); group 3, stromal cells+/Tumor+ (20/48 cases); group 4, stromal cells-/Tumor- (3/48 cases). Immunostaining of L1CAM-positive stromal cells of the TME with CD163 and alpha-SMA showed that both macrophages (CD163-positive) and fibroblasts (alpha-SMA-positive) were involved in L1CAM expression. L1CAM expression in stromal cells was also associated with the acquisition of a motile phenotype by tumor cells: among 20 CRCs with budding margins, 17 (85%) were characterized by L1CAM expression in peritumoral infiltrating cells. In this study, L1CAM expression in the peritumoral stroma was not limited to stromal cells, being also expressed in cells of the enteric nervous system. The strong immunoreactivity of neural structures for L1CAM allowed a better identification of perineural invasion in CRC. According to our preliminary results, the study of L1CAM expression in the peritumoral infiltrating cells in CRC patients could represent a valid tool able to stratify patients with colorectal cancer regarding the possible occurrence of metastasis and recurrence.

Doi: 10.48286/aro.2023.76

Impact statement: The positivity of L1CAM in patients with CRC and in CAF as well as 62 a valid help to stratify patients for the possible onset of metastases and recurrences 63 could be a valid study potential for improving the therapeutic approach.

Key words: *L1CAM; colorectal cancer; immunohistochemistry; cancer-associated fibroblasts.*

Received: Jul 28, 2023/**Accepted**: Nov 2, 2023
Published: Dec 22, 2023

INTRODUCTION

In recent decades, remarkable revolutions have taken place in the field of carcinogenesis. Recent reports on the tumor microenvironment (TME) have completely reformed the classical view of cancer as simply a tumor cell-centric disease. According to the "seed and soil" theory, first proposed by Steven Paget in 1889 (1) and recently revisited according to the more recent data on the tumor-stroma interactions (2), tumor insurgence and progression are governed by the strict interactions between cancer cells (the seed) and the surrounding TME of the host organ (the soil) (3). The TME mainly consists of tumor cells, stromal cells, immune cells, intratumoral and peritumoral blood vessels, and extracellular matrix (ECM) (4). The ECM comprises an intricate network of extracellular proteins, glycoproteins, and proteoglycans that assemble into supramolecular aggregates, including fibrils. These structures undergo substantial remodeling in response to the influence exerted by primary solid tumors (5). In the peritumoral stroma, resident fibrocytes and fibroblasts differentiate into cancer-associated fibroblasts (CAFs) which modify the ECM, increasing tissue stiffness and favoring the insurgence of desmoplasia (6).

In colorectal cancer (CRC), tumor cells undergo epithelial-to-mesenchymal transition and become invasive tumor cells (7). During this process, cells of the TME play a fundamental role in regulating the acquisition of a motile phenotype by cancer cells (8, 9). Several resident cell types embedded in the TME may interact with invasive tumor cells, engaging with them a cross-talk mediated by multiple membrane and surface adhesion molecules (10). Multiple cell types of the TME have been involved in the induction of cancer cell invasion: tumor-associated macrophages (TAMs) (10, 11), cancer-associated fibroblasts (CAFs) (12, 13), resident-activated fibroblasts (RAFs) (14), endothelial cells favoring intravasation (15) and Schwann cells which favor perineural invasion (16, 17).

Regarding CAFs, they are composed of a heterogeneous population of cells (18). As a consequence, no reliable immunohistochemical markers for all the subtypes of CAFs are available (19). CD10 has been proposed as a marker of a subset of CAFs (20). Collagen I, PDGFR-β, and α-SMA have been proposed as molecular markers of other subpopulations of CAFs (21). The immunoreactivity of CAFs for markers, like α-SMA, CD10, podoplanin, and fibroblast-specific protein 1 (FSP1), has been correlated with the insurgence of lymph node metastases in colorectal cancer (22). A recent proteomic analysis of CAFs has revealed significant differences between CAFs and normal fibroblasts regarding their protein expression profiles, with 145 differentially expressed proteins (23).

Recently, colorectal neural cells, belonging to the enteric nervous system (ENS) have been involved in the physical guidance of colon cancer cells during their invasion (24). The components of ENS, enteric neurons and enteric glial cells, give rise to the digestive neuronal-glial-epithelial unit, an important entity in intestinal health and disease (25). In particular, enteric neurons and enteric glia have been reported to be involved in the proliferation and migration of tumor cells in CRC (26, 17).

The aim of this work was to analyze in a series of colorectal cancers the expression of the cell adhesion molecule L1 (L1CAM) in the tumor microenvironment of CRC, in order to better clarify the role the TME in its insurgence and progression.

PATIENTS AND METHODS

A retrospective study was conducted on formalin-fixed paraffin-embedded tissue samples of CRC using conventional techniques. The objective was to examine the immunoreactivity of tumor cells for L1CAM in the microenvironment of CRC. The biopsy samples and tissue resections of CRC were obtained at the Division of Pathology of the University Hospital of Cagliari.

In the present study, 48 patients affected by colorectal adenocarcinoma, ranging in age from 38 to 88

Table 1. *Clinical, histological, and immunohistochemical data regarding 48 cases of colorectal cancer*

CASE	AGE	GENDER	TUMOR GRADE	L1CAM CAF	BUDDING MARGINS	L1CAM TUMOR
1	66	F	G1	-	-	-
2	59	M	G2	-	-	-
3	61	F	G3	+	-	-
4	65	M	G2	+	-	-
5	51	F	G1	-	-	+
6	43	F	G1	+	-	+
7	67	F	G2	+	+	+
8	67	M	G3	+	-	-
9	81	M	G3	+	+	+
10	83	F	G2	+	+	+
11	82	F	G3	-	-	-
12	81	F	G2	+	-	-
13	66	F	G2	+	++	+
14	82	M	G2	+	-	+
15	88	F	G2	+	-	-
16	80	M	G1	+	+	+
17	79	M	G3	+	-	-
18	49	M	G2	+	+	+
19	71	M	G3	+	-	-
20	59	M	G1	-	-	+
21	38	F	G3	+	+	+
22	70	M	G1	-	+	+
23	79	F	G2	+	+	+
24	75	F	G2	+	-	-
25	55	F	G1	-	-	+
26	49	F	G2	-	-	+
27	70	M	G2	-	-	+
28	74	M	G2	-	-	+
29	84	F	G2	+	+	+
30	47	M	G2	+	-	-
31	65	M	G2	+	+	+
32	81	M	G2	+	-	-
33	40	F	G2	+	+	+
34	84	M	G1	+	-	-
35	82	F	G2	+	-	-
36	67	M	G2	+	+	+
37	78	M	G2	+	+	+
38	70	M	G2	+	+	+
39	77	M	G1	+	-	-
40	85	M	G2	+	-	+
41	81	F	G2	+	-	-
42	54	M	G1	+	-	+
43	58	M	G2	+	-	-
44	74	M	G2	+	+	+
45	74	F	G2	+	+	+
46	69	M	G2	+	+	+
47	78	M	G2	-	+	+
48	65	M	G2	+	+	+

years, 20 females and 28 males, were analyzed. The clinical and pathological data are reported in **Table 1**.

Three- four micron-thick sections were stained with hematoxylin and eosin (H&E) and immunostained with a mouse monoclonal antibody (Sigma-Aldrich, clone UJ127) against L1CAM (mouse IgG1 isotype). The ultra-View Universal DAB Detection Kit was used for detecting primary antibodies. The sections were automatically dewaxed and rehydrated with concentrated EZ Prep (10X) (cat. no. 950-102 / 05279771001) and pretreated with the recovery of the heat-induced epitope in ULTRA Cell Conditioning Solution (ULTRA CC1) (cat. 950-224 / 0524569001), for 64 minutes at 95°, to follow the slides were incubated for 20 minutes at room temperature at 1:100 dilution of the monoclonal anti-L1CAM primary antibody. Nervous structures were utilized as internal positive controls for L1CAM. As appropriate negative controls, tissue sections were processed omitting the primary antibody for L1CAM.

L1CAM expression was evaluated in a blinded fashion, without knowledge of clinical and pathological information. The sections were analyzed at high magnification to assess the positivity of L1CAM immunostaining in the microenvironment of CRC and in tumor cells. We regarded the staining as positive in cases with cytoplasmic and/or cell membrane positivity of cells of the TME. No nuclear reactivity for L1CAM was detected in infiltrating cells of the TME. The χ^2 test was used to examine the association between L1CAM expression in spindle cells of the TME and clinic-pathological characteristics of patients, including age, gender, and degree of differentiation (grading) of tumor cells. The small size of

the Cohort of CRC patients analyzed is a limit of this study. As a consequence, our findings regarding the correlation of L1CAM expression in the TME with the clinical-pathological findings should be considered as preliminary data. All procedures were performed according to the Ethical National standards of the responsible committee on human experimentation and approved by the Ethic Human Studies Committee of the University Medical Center of Cagliari (N. PG/2020/10912).

RESULTS

The study group included 28 male and 20 female patients ranging from 38 to 88 years of age (mean, 68 years) affected by CRC. Pertinent clinical, histological, and immunohistochemical findings regarding L1CAM expression in infiltrating spindle cells in the microenvironment of CRC are presented in **Table 1**. At histology, 10 tumors were slow-grade adenocarcinomas (G1), 31 showed a moderate grade of differentiation (G2) and the remaining 7 cases were classified as high-grade adenocarcinomas (G3). L1CAM expression was observed in the TME in 37 out of 48 cases (77%) (**Table 1**). In positive cases, immunoreactivity for L1CAM was restricted to the cell membrane and to the cytoplasm of spindle cells the TME (**Figure 1**), in the absence of any nuclear immunostaining. No reactivity was detected in in the TME in the remaining 11 tumors (23%). A marked interindividual variability was observed regarding the degree of immunoreactivity for L1CAM in the peritumoral stroma in the 22 positive cases, ranging from focal staining, involving less than 10% of cells of the TME (**Figure 2**) to diffuse

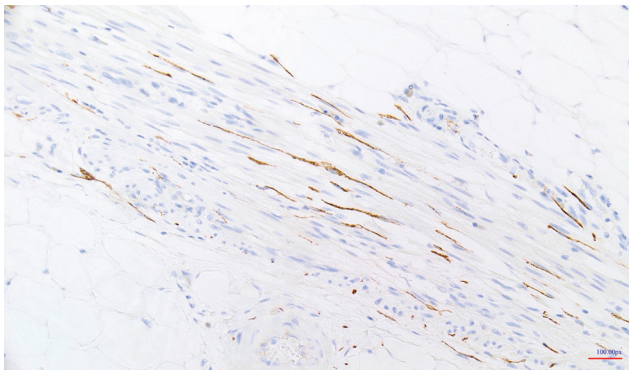

Figure 1. *Typical L1CAM expression in infiltrating stromal cells of the microenvironment.*

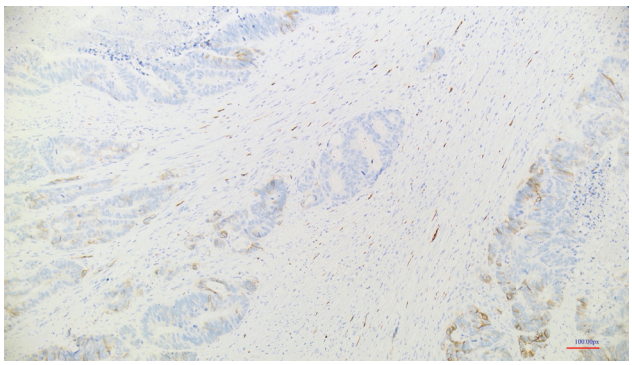

Figure 2. *Focal reactivity for L1CAM in spindle cells of the TME.*

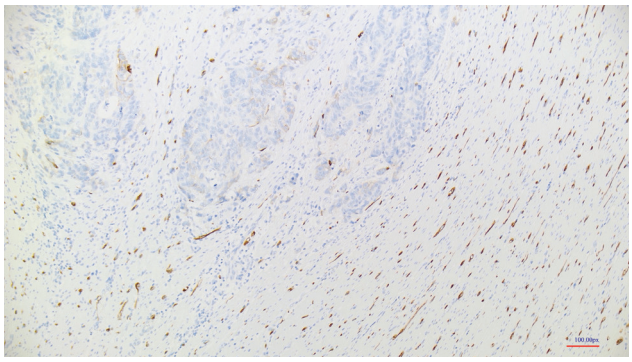

Figure 3. *Diffuse immunostaining for L1CAM in tumor-infiltrating spindle cells in the TME of colorectal adenocarcinoma.*

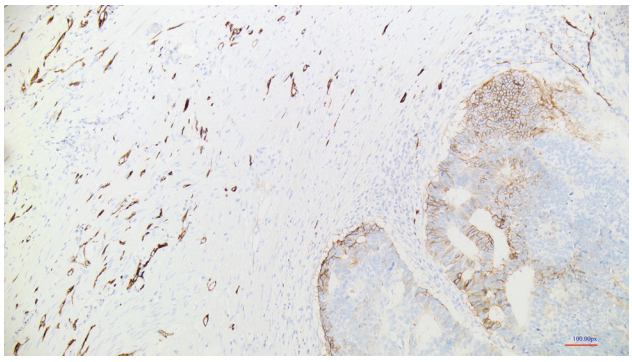

Figure 4. *L1CAM expression both infiltrating spindle stromal cells and in tumor cells.*

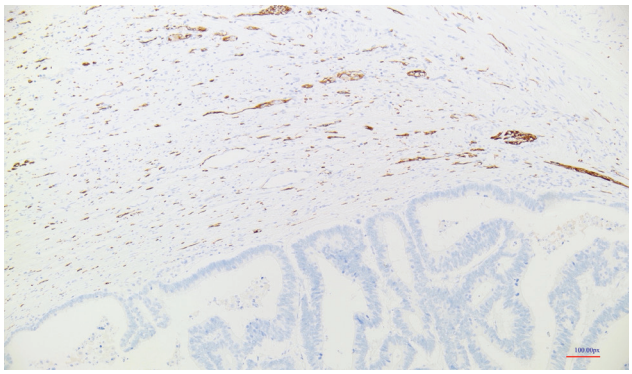

Figure 5. *L1CAM expression is restricted to stromal cells of the microenvironment in the absence of any expression in tumor cells.*

immunoreactivity (**Figure 3**). In 22 out of 30 cases showing expression of L1CAM in the TME, the adhesion molecule was also expressed in tumor cells (**Figure 4**). In the remaining 15 cases, L1CAM expression was restricted to stromal tumor-infiltrating cells (**Figure 5**). When evaluating the expression of spindle infiltrating cells and tumor cells for L1CAM, four subgroups were identified:

Group 1: stromal cells+/Tumor cells+ (22 cases).
Group 2: stromal cells+/Tumor cells- (15 cases).
Group 3: stromal cells-/Tumor cells+ (8 cases).
Group 4: stromal cells-/Tumor cells- (3 cases).

A strict association was found between the presence of budding margins and the expression of L1CAM in the peritumoral stromal cells. Among the 30 CRCs with L1CAM-positive cells of the CRC microenvironment, 20 were characterized by budding margins (67%). Among the 20 cases of CRC characterized by budding margins, 18 showed immunoreactivity for L1CAM in the TME (90%). We also observed a higher concentration of L1CAM-positive infiltrating spindle cells in close proximity to tumor cells acquiring a motile phenotype and infiltrating the peritumoral microenvironment (**Figure 6**).

A correlation was observed between the degree of differentiation of colorectal cancer cells and L1CAM expression in the TME. Among the 7 high-grade cases of CRC (G3), 6 showed immunoreactivity of infiltrating stromal cells for L1CAM (86%). L1CAM-expressing tumor-infiltrating cells were unevenly distributed within the tumor microenvironment. Areas with diffuse L1CAM-reactivity of stromal cells were observed adjacent to areas negative for the cell adhesion molecule. Within the tumor microenvironment, L1CAM expression was not restricted to stromal cells, being also expressed in the structures of the enteric nervous system of the intestinal wall. The strong reactivity of nervous structures for L1CAM allowed a better identification of perineural invasion occurring along extrinsic nerves, with Schwann cells expressing L1CAM and providing physical guidance for tumor cells (**Figure 7**). In order to better clarify the nature of L1CAM-positive stromal cells of the TME, L1CAM immunostaining was compared with immunostaining for anti-smooth muscle actin antibodies (SMA), CD163 and Pan-CK in the same histological field (**Fig. 8**). In the peritumoral stroma, L1CAM was expressed in the majority of spindle cells with morphological features suggestive CAFs (**Figure 8A**). Focal reactivity for L1CAM was present also in tumor cells. In **Figure 8B**, CD163 immunostaining revealed the presence of a high number of tumor-associated monocytes/macrophages (TAMs) in the peritumoral stromal reaction, including round and spindle cells. No expression of CD163 was detected in tumor cells. Alpha-SMA reactivity characterized the vast majority of spindle cells surrounding tumor cells, putatively representing activated fibroblasts associated to CRC (**Figure 8C**). Pan-CK stain of the same specimen evienced immunoreactivity for cytokeratins the majority of tumor cells. No immunostaining for CK was found in the stromal cells of the tumor microenvironment (**Figure 8D**).

We investigated the dependence between the presence of budding margins and the expression of L1CAM in CAFs using a chi-squared test of independence with the null hypothesis that the proportion of samples presenting budding margins is independent of the expression of L1CAM stromal infiltrating cells.

Our results show $X2 (1, N = 48) = 3.24$, $p = .0072$, then we cannot reject the null hypothesis, so we cannot rule out the possibility that the 2 variables are independent.

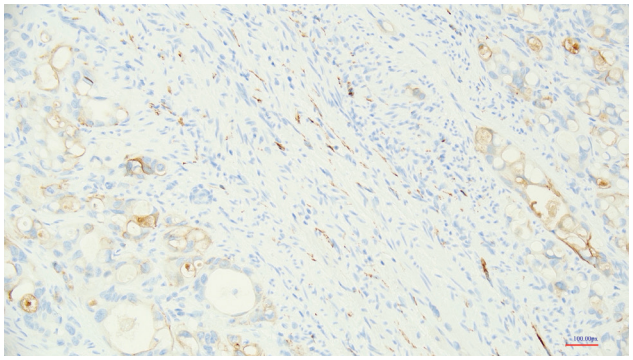

Figure 6. *High expression in peritumoral spindle stromal cells in close proximity to cancer cells infiltrating the peritumoral stroma.*

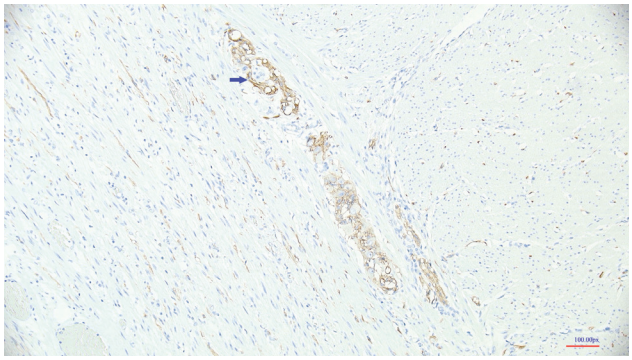

Figure 7. *Perineural invasion: L1CAM-positive Schwann cells provide physical guidance to cancer cells.*

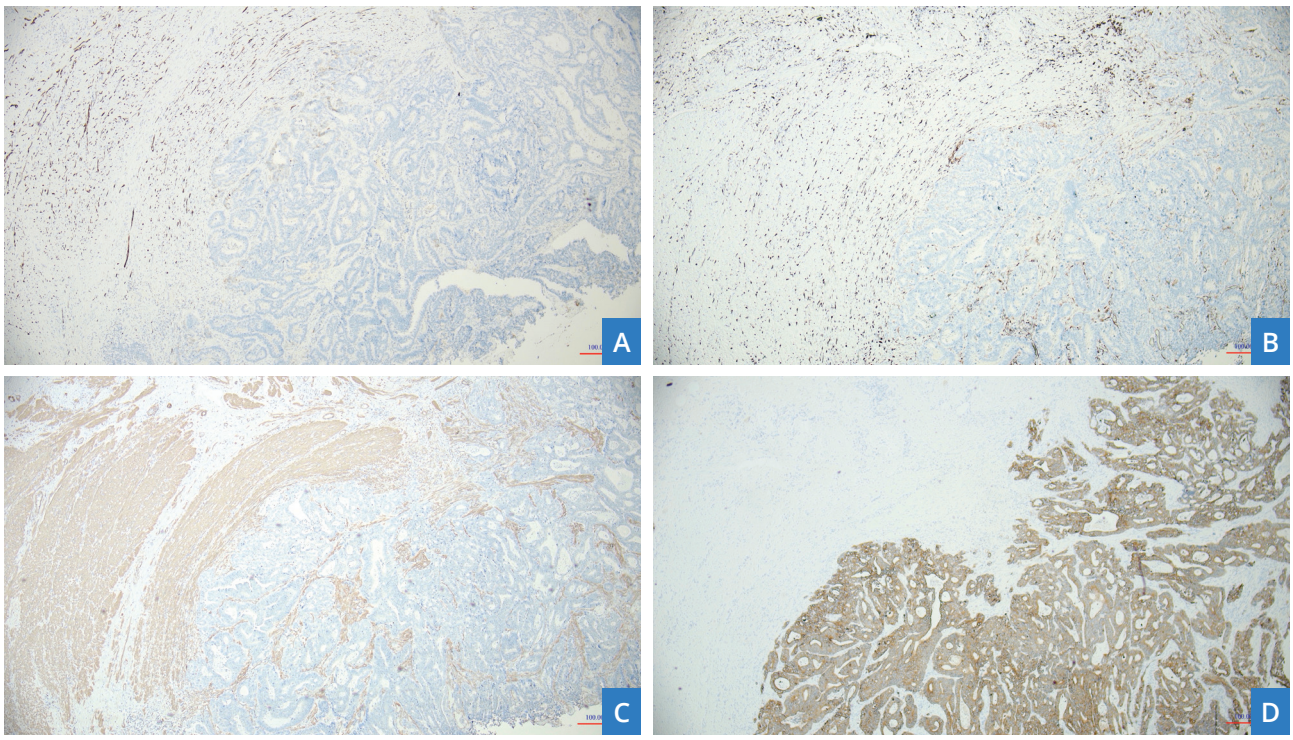

Figure 8. *L1CAM (**A**), CD163 (**B**), SMA (**C**) and Pan-CK (**D**). Expression in the same histological field. (**A**) L1CAM is expressed in the majority of spindle cells of the peritumoral stromal reaction. Focal reactivity for L1CAM is present also in tumor cells. (**B**) CD163 immunostaining reveals the presence of a high number of tumor-associated monocytes/macrophages in the peritumoral stromal reaction, including round and spindle cells. No expression of CD163 is detected in tumor cells. (**C**) alpha-SMA reactivity characterizes the vast majority of spindle cells surrounding tumor cells. (**D**) CK20 stains the majority of tumor cells. No immunostaining for CK20 is found in the tumor microenvironment.*

DISCUSSION

Fibroblasts of the TME, better known as cancer-associated fibroblasts (CAFs), are key stroma cells that play a major role in tumor insurgence, progression, promoting tumor cell growth, the epithelial-mesenchymal transition of tumor cells, and metastasis (27, 28). Regarding their origin, CAFs predominantly originate from multiple local mesenchymal cells, including fibrocytes, peritumoral fibroblasts, mesothelial cells, vascular smooth muscle cells, pericytes, and endothelial cells of the peritumoral vessels (29). Through the process of endothelial mesenchymal transition (EndMT), endothelial cells may detach from neighboring cells and acquire a migratory phenotype, eventually originating CAFs (30). CAFs might also originate from circulating precursors, such as circulating fibrocytes, a leukocyte subpopulation with a peculiar immunohistochemical marking originating from hematopoietic stem cells (31). Fibrocytes are characterized by the expression of fibroblastic markers (collagen type I, vimentin, fibronectin) associated with leukocyte markers such as CD45 and CD34, in the absence of CD15 expression (32).

Very recently, multiple subpopulations of CAFs have been described: Inflammatory (iCAFs), myofibroblastic (myCAFs), antigen-presenting (apCAFs), CAFs with a highly activated metabolic state (meCAFs), and complement-secreting (csCAFs) (33). These findings support the hypothesis that CAFs consist of multiple plastic and heterogeneous population that participate in bidirectional signaling pathways between the multiple cell types that give rise to the TME (34). The heterogeneity of CAFs has been recently confirmed by the report of a subpopulation of CAFs producing prolargin, a powerful tumor-suppressor that inhibits several pro-angiogenic proteins resulting in tumor suppression and better outcome (35).

Our understanding of the role of CAFs in tumor progression has been significantly enhanced with the discovery that the decreased expression of certain microRNAs secreted by CAFs is linked to unfavorable clinical outcomes (36). The balance between GREM1 and ISLR, two CAF-specific genes involved in the bone morphogenic protein (BMP) signaling pathway in the TME, plays a major role in driving tumor progression (37). The expression of

high levels of GREM1 is associated with poor survival whereas high ISLR expression is associated with favorable outcomes in colorectal cancer (38).

In this study, we analyzed the immunoreactivity of stromal cells of the microenvironment of CRC, including cancer-associated fibroblasts (CAFs), towards the L1 cell adhesion molecule (L1CAM). L1CAM is a cell adhesion molecule initially identified in neurons of the cerebral cortex and found in various organs during fetal development, including the gastrointestinal tract and associated glands (39), kidneys (40), and the spinal cord (41). In recent years, L1CAM expression has been reported in multiple tumors, in which it is involved in cell motility, proliferation, invasion, and resistance to chemotherapy (42). Moreover, L1CAM immunostaining has been associated with poor prognosis, poor survival outcomes, and adverse clinical course (43). A recent study from our group confirmed that L1CAM is highly expressed in tumor cells in a subset of CRCs (44).

Here we first demonstrate that L1CAM is expressed also in stromal infiltrating cells in most of the CRCs analyzed (37/48). L1CAM expression in the TME of CRC was not strictly related to its expression in tumor cells. Among the 37 cases characterized by L1CAM expression in stromal cells in the microenvironment, only 22 (46%) showed immunoreactivity for L1CAM even in tumor cells. On the other hand, only 22/30 CRCs with L1CAM expression in tumor cells showed immunostaining for L1CAM in the peritumoral stroma. Four groups were identified by the evaluation of L1CAM expression in tumor infiltrating stromal cells and cancer cells. Group 1: stromal cells+ and Tumor cells+ (22 cases); Group 2: stromal cells+ and Tumor cells- (15 cases); Group 3: stromal cells- and Tumor cells+ (8 cases); Group 4: stromal cells- and Tumor cells- (3 cases).

L1CAM reactivity was not strictly related to the tumor grade: L1CAM-positive stromal cells were observed in G1, and G2 as well in G3 CRCs. On the other hand, when taking into consideration the 8 G3 cases, 6 cases (75%) showed immunostaining for L1CAM in stromal cells of the TME. This finding suggests the existence of some relationship between tumor differentiation and L1CAM expression in peritumoral stromal cells, which should be re-evaluated in further studies carried out on a larger series of CRCs.

Another interesting finding emerging from our study is the relationship between L1CAM expression in infiltrating stromal cells and the presence of infiltrative "budding" margins in the tumor mass. Among the 20 CRCs with budding margins, 18 (90%) were characterized by the immunostaining for L1CAM in infiltrating stromal cells. Moreover, among the 22 CRCs with reactivity for L1CAM in the TME, 18 (47%) showed budding margins. Looking at the 3 cases with no expression of L1CAM both in infiltrating stromal cells and in tumor cells (group 4), in 3 out of 3 cases we did not observe budding margins. When taking into consideration the 22 cases with L1CAM reactivity both in peritumoral stromal cells and in tumor cells (group 1), 18 out of 22 cases (90%) showed the presence of budding margins. Al these findings taken together indicate some association between the acquiring of a motile phenotype by cancer cells and the expression of L1CAM in tumor-infiltrating stromal cells and/or in tumor cells. The evaluation of L1CAM expression in both members of the TME, stromal cells and tumor cells, according to our work, might improve the significance of L1CAM expression in CRC, allowing a better stratification of patients with a more aggressive phenotype and worse clinical behavior.

CONCLUSIONS

This work lays stress on two major points: i) the relevant role played by the tumor microenvironment, and in particular by the cancer-associated fibroblasts and tumor-associated macrophages, in the insurgence and progression of colorectal cancer; ii) the role of the expression of L1CAM both in peritumoral fibroblasts and in tumor cells in favoring the acquisition of a motile phenotype by cancer cells, ending with the insurgence of budding margins. According to our preliminary findings, the study of L1CAM expression in infiltrating stromal cells of the microenvironment in CRC patients might increase the ability of oncologists to stratify patients affected by colorectal cancer (44) regarding the possible insurgence of metastases and recurrences. These findings taken together, suggest the relevant role played by the immunohistochemical detection of L1CAM in every CRC and the evaluation of L1CAM expression both in tumor cells and in tumor-infiltranting stromal cells. Moreover, according to our findings, we believe that with the use of CAF-specific antibodies, the identification of CAFs should be further investigated as a potential factor influencing clinical outcomes during immunotherapy. This would be particularly relevant in colorectal tumors

in order to better identify patients who are more likely to benefit from such a treatment approach (45). The main limitation of the current study is represented by the small size of the cohort of CRC patients analyzed. Further studies with larger prospective recruitment will better clarify the role of L1CAM expression in stromal cells of the CRC environment in its insurgence and progression.

ACKNOWLEDGMENTS

Flaviana Cau is a student of the International Ph.D. in "Innovation Sciences and Technologies" at the University of Cagliari, Cagliari, Italy.

COMPLIANCE WITH ETHICAL STANDARDS

Fundings

There was no institutional or private funding for this article.

Conflicts of Interests

The Authors have declared no conflict of interests.

Authors' Contributions

All the Authors listed in the manuscript have contributed to the conception and planning of the work, through the acquisition of the patient's clinical data, choice of cases, preparation procedure of the preparations, and interpretation of the results. All the Authors participated in the drafting of the manuscript and in the definitive approval of the version to be published.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethical approval

Human studies and subjects

All procedures were performed according to the ethical national standards of the responsible committee on human experimentation and approved by the Ethic Human Studies Committee of the University Medical Center of Cagliari (N. PG/2020/10914).

Animal studies

N/A.

Publication ethics

Plagiarism

The contents of the article are original and any overlaps with other articles are by the Authors themselves and appropriately cited.

Data falsification and fabrication

All the data correspond to the real.

REFERENCES

1. Ribatti D, Mangialardi G, Vacca A. Stephen Paget and the 'seed and soil' theory of metastatic dissemination. Clin Exp Med. 2006 Dec;6(4):145-9. doi: 10.1007/s10238-006-0117-4.
2. Langley RR, Fidler IJ. The seed and soil hypothesis revisited--the role of tumor-stroma interactions in metastasis to different organs. Int J Cancer. 2011;128(11):2527-35. doi: 10.1002/ijc.26031.
3. Akhtar M, Haider A, Rashid S, Al-Nabet ADMH. Paget's "Seed and Soil" Theory of Cancer Metastasis: An Idea Whose Time has Come. Adv Anat Pathol. 2019;26(1):69-74. doi: 10.1097/PAP.0000000000000219.
4. Fidler IJ, Poste G. The "seed and soil" hypothesis revisited. Lancet Oncol. 2008;9(8):808. doi: 10.1016/S1470-2045(08)70201-8.
5. Eble JA, Niland S. The extracellular matrix in tumor progression and metastasis. Clin Exp Metastasis. 2019;36(3):171-98. doi: 10.1007/s10585-019-09966-1.
6. Erdogan B, Webb DJ. Cancer-associated fibroblasts modulate growth factor signaling and extracellular matrix remodeling to regulate tumor metastasis. Biochem Soc Trans. 2017;45(1):229-36. doi: 10.1042/BST20160387.
7. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012;21(3):309-22. doi: 10.1016/j.ccr.2012.02.022.
8. Friedl P, Wolf K. Plasticity of cell migration: a multiscale tuning model. J Cell Biol. 2010;188(1):11-9. doi: 10.1083/jcb.200909003.
9. Sandberg TP, Stuart MPME, Oosting J, Tollenaar RAEM, Sier CFM, Mesker WE. Increased expression of cancer-associated fibroblast markers at the invasive front and its association with tumor-stroma ratio in colorectal cancer. BMC Cancer. 2019;19(1):284. doi: 10.1186/s12885-019-5462-2.

10. Roh-Johnson M, Bravo-Cordero JJ, Patsialou A, Sharma VP, Guo P, Liu H et al. Macrophage contact induces RhoA GTPase signaling to trigger tumor cell intravasation. Oncogene. 2014;33(33):4203-12. doi: 10.1038/onc.2013.377.
11. Giacomelli M, Monti M, Pezzola DC, Lonardi S, Bugatti M, Missale F, et al. Immuno-Contexture and Immune Checkpoint Molecule Expression in Mismatch Repair Proficient Colorectal Carcinoma. Cancers (Basel). 2023;15(12):3097. doi: 10.3390/cancers15123097.
12. Labernadie A, Kato T, Brugués A, Serra-Picamal X, Derzsi S, Arwert E, et al. A mechanically active heterotypic E-cadherin/N-cadherin adhesion enables fibroblasts to drive cancer cell invasion. Nat Cell Biol. 2017;19(3):224-37. doi: 10.1038/ncb3478.
13. Shin N, Son GM, Shin DH, Kwon MS, Park BS, Kim HS, et al. Cancer-Associated Fibroblasts and Desmoplastic Reactions Related to Cancer Invasiveness in Patients With Colorectal Cancer. Ann Coloproctol. 2019;35(1):36-46. doi: 10.3393/ac.2018.09.10.
14. Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, et al. A framework for advancing our understanding of cancer-associated fibroblasts. Nat Rev Cancer. 2020;20(3):174-86. doi: 10.1038/s41568-019-0238-1.
15. Yeon JH, Jeong HE, Seo H, Cho S, Kim K, Na D, et al. Cancer-derived exosomes trigger endothelial to mesenchymal transition followed by the induction of cancer-associated fibroblasts. Acta Biomater. 2018;76:146-53. doi: 10.1016/j.actbio.2018.07.001.
16. Deborde S, Omelchenko T, Lyubchik A, Zhou Y, He S, McNamara WF, et al. Schwann cells induce cancer cell dispersion and invasion. J Clin Invest. 2016; 126(4):1538-54. doi: 10.1172/JCI82658.
17. Duchalais E, Guilly C, Nedellec S, Touvron M, Bassard A, et al. Colorectal cancer cells adhere to and migrate along neurons of the enteric nervous system. Cell Mol Gastroenterol Hepatol. 2018; 5: 31-49. doi: 10.1016/j.jcmgh.2017.10.002.
18. Nurmik M, Ullmann P, Rodriguez F, Haan S, Letellier E. In search of definitions: Cancer-associated fibroblasts and their markers. Int J Cancer. 2020; 146(4):895-905. doi: 10.1002/ijc.32193.
19. Deng L, Jiang N, Zeng J, Wang Y, Cui H. The Versatile Roles of Cancer-Associated Fibroblasts in Colorectal Cancer and Therapeutic Implications. Front Cell Dev Biol 2021: 1;9:733270. doi: 10.3389/fcell.2021.733270.
20. Zhu Y, Zheng JJ, Yang F, Nie QQ, Zhu ZL, Deng H. [Expression of CD10 in cancer-associated fibroblasts and its effect on initiation and progression of colorectal carcinoma]. Zhonghua Bing Li Xue Za Zhi 2016;45(12):859-65. Chinese. doi: 10.3760/cma.j.issn.0529-5807.2016.12.009.
21. Nishishita R, Morohashi S, Seino H, Wu Y, Yoshizawa T, Haga T, et al. Expression of cancer-associated fibroblast markers in advanced colorectal cancer. Oncol Lett. 2018;15(5):6195-202. doi: 10.3892/ol.2018.8097.
22. Sugai T, Uesugi N, Kitada Y, Yamada N, Osakabe M, Eizuka M, et al. Analysis of the expression of cancer-associated fibroblast- and EMT-related proteins in submucosal invasive colorectal cancer. J Cancer. 2018;9(15):2702-712. doi: 10.7150/jca.25646.
23. Atanasova VS, Tiefenbacher A, Clement J, Wöran K, Bergmann M, Dolznig H, et al. Identification of proteome and secretome signatures in primary colorectal cancer-associated fibroblasts. Annals of Oncology. 2020;31:S445. doi: 10.1016/j.annonc.2020.08.593.
24. Duchalais E, Machairas N, Kelley SR, Landmann RG, Merchea A, Colibaseanu DT, et al. Does prolonged operative time impact postoperative morbidity in patients undergoing robotic-assisted rectal resection for cancer? Surg Endosc. 2018;32(8):3659-66. doi: 10.1007/s00464-018-6098-z.
25. Neunlist M, Van Landeghem L, Mahé MM, Derkinderen P, Des Varannes SB, Rolli-Derkinderen M. The digestive neuronal-glial-epithelial unit: a new actor in gut health and disease. Nat Rev Gastroenterol Hepatol. 2013;10(2):90-100. doi: 10.1038/nrgastro.2012.221.
26. Ueno H, Shirouzu K, Shimazaki H, Kawachi H, Eishi Y, Ajioka Y, et al. Study Group for Perineural Invasion projected by the Japanese Society for Cancer of the Colon and Rectum (JSCCR). Histogenesis and prognostic value of myenteric spread in colorectal cancer: a Japanese multi-institutional study. J Gastroenterol. 2014;49(3):400-7. doi: 10.1007/s10147-019-01485-z.
27. Wright K, Ly T, Kriet M, Czirok A, Thomas SM. Cancer-Associated Fibroblasts: Master Tumor Microenvironment Modifiers. Cancers (Basel). 2023;15(6):1899. doi: 10.3390/cancers15061899.

28. Kalluri R. The biology and function of fibroblasts in cancer. Nat Rev Cancer. 2016;16(9):582-98. doi: 10.1038/nrc.2016.73.
29. Arina A, Idel C, Hyjek EM, Alegre ML, Wang Y, Bindokas VP, et al. Tumor-associated fibroblasts predominantly come from local and not circulating precursors. Proc Natl Acad Sci USA. 2016;113(27):7551-6. doi: 10.1073/pnas.1600363113.
30. Choi KJ, Nam JK, Kim JH, Choi SH, Lee YJ. Endothelial-to-mesenchymal transition in anticancer therapy and normal tissue damage. Exp Mol Med. 2020;52(5):781-92. doi: 10.1038/s12276-020-0439-4.
31. Cao T, Rajasingh S, Rajasingh J. Circulating fibrocytes serve as a marker for clinical diagnosis. Ann Transl Med. 2016;4(Suppl 1):S38. doi: 10.21037/atm.2016.10.26.
32. Ghanem M, Homps-Legrand M, Garnier M, Morer L, Goletto T, Frija-Masson J, et al. Blood fibrocytes are associated with severity and prognosis in COVID-19 pneumonia. Am J Physiol Lung Cell Mol Physiol. 2021;321(5):L847-L858. doi: 10.1152/ajplung.00105.2021.
33. Li X, Zhou J, Wang X, Li C, Ma Z, Wan Q, et al. Pancreatic cancer and fibrosis: Targeting metabolic reprogramming and crosstalk of cancer-associated fibroblasts in the tumor microenvironment. Front Immunol. 2023;14:1152312. doi: 10.3389/fimmu.2023.1152312.
34. Li Z, Sun C, Qin Z. Metabolic reprogramming of cancer-associated fibroblasts and its effect on cancer cell reprogramming. Theranostics. 2021;11(17):8322-36. doi: 10.7150/thno.62378.
35. Chiavarina B, Ronca R, Otaka Y, Sutton RB, Rezzola S, Yokobori T, et al. Fibroblast-derived prolargin is a tumor suppressor in hepatocellular carcinoma. Oncogene. 2022;41(10):1410-20. doi: 10.1038/s41388-021-02171-z.
36. Eun JW, Ahn HR, Baek GO, Yoon MG, Son JA, Weon JH, et al. Aberrantly Expressed MicroRNAs in Cancer-Associated Fibroblasts and Their Target Oncogenic Signatures in Hepatocellular Carcinoma. Int J Mol Sci. 2023;24(5):4272. doi: 10.3390/ijms24054272.
37. Ehata S, Miyazono K. Bone Morphogenetic Protein Signaling in Cancer; Some Topics in the Recent 10 Years. Front Cell Dev Biol. 2022;10:883523. doi: 10.3389/fcell.2022.883523.
38. Kobayashi H, Gieniec KA, Wright JA, Wang T, Asai N, Mizutani Y, et al. The Balance of Stromal BMP Signaling Mediated by GREM1 and ISLR Drives Colorectal Carcinogenesis. Gastroenterology. 2021;160(4):1224-39.e30. doi: 10.1053/j.gastro.2020.11.011.
39. Cau F, Piras M, Congiu T, Murru R, Aimola V, Cerrone G, et al. L1CAM expression in human gastrointestinal tract development: from tongue to colon-rectum. J Public Health Res. 2023; 16;12(2):22799036231165624. doi: 10.1177/22799036231165624.
40. Cau F, Gerosa C, Murru R, Pichiri G, Coni P, Piras M, et al. Interindividual variability in L1CAM expression in the human kidney during development: are there implications for fetal programming of kidney diseases presenting in adulthood? Eur Rev Med Pharmacol Sci. 2022;26(12):4346-53. doi: 10.26355/eurrev_202206_29073.
41. Cau F, Fanni D, Manchia M, Gerosa C, Piras M, Murru R,et al. Expression of L1 Cell Adhesion Molecule (L1CAM) in extracellular vesicle in the human spinal cord during development. European Review for Medical and Pharmacological Sciences. 2022;26(17):6273-82. doi: 10.26355/eurrev_202209_29651.
42. Kiefel H, Pfeifer M, Bondong S, Hazin J, Altevogt P. Linking L1CAM-mediated signaling to NF-κB activation. Trends Mol Med. 2011;17(4):178-87. doi: 10.1016/j.molmed.2010.11.005.
43. Guo M, Gong H, Nie D, Li Z. High. L1CAM expression predicts poor prognosis of patients with endometrial cancer: A systematic review and meta-analysis. Medicine (Baltimore). 2021;100(13):e25330. doi: 10.1097/MD.0000000000025330.
44. Cau F, Gerosa C, Ziranu P, Pretta A, Murru R, Fraschini M, Piras M, et al. L1CAM expression identifies different colorectal tumor subgroups. Annals of Research in Oncology. 2023;3(2):75-85. doi: 10.48286/aro.2023.69.
45. Ziranu P, Pretta A, Pozzari M, Maccioni A, Badiali M, Fanni D. et al. CDX-2 expression correlates with clinical outcomes in MSI-H metastatic colorectal cancer patients receiving immune checkpoint inhibitors. Sci Rep. 2023;13(1):4397. doi: 10.1038/s41598-023-31538-3.