

RESEARCH ARTICLE

L1CAM EXPRESSION IDENTIFIES DIFFERENT COLORECTAL TUMOR SUBGROUPS

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ABSTRACT: Neural cell adhesion molecule L1 (L1CAM) is a stem cell marker belonging to the immunoglobulin superfamily of cell adhesion molecules (IgCAMs). L1CAM shows multiple functions, depending on its homophilic or heterophilic interactions with other L1CAM molecules or with cell-matrix components. Previous studies have shown that L1CAM is aberrantly activated in colon cancer development and progression, being associated with metastasis. The present study was aimed to assess, using immunohistochemistry, the expression pattern of L1CAM in colorectal cancer (CRC), in order to correlate L1CAM expression with the aggressivity of tumor cells. To this end, formalin-fixed paraffin-embedded tissue samples from 51 patients affected by CRC, ranging in age from 38 to 88 years, 21 females and 30 males, were analyzed. The most important finding emerging from our work is the marked interindividual variability in L1CAM immunoreactivity in CRC, with 31 out of 51 (61%) cases expressing L1CAM. In positive cases, the expression of L1CAM in tumor cells ranged from mild immunostaining in 22 cases (score 1), to moderate reactivity in 6 (score 2) to strong and diffuse expression in 3 tumors (score 3). This is a new finding regarding L1CAM expression in CRC, which evidences that in a large cohort (39%) of CRC, tumor cells are not immunoreactive for this cell adhesion molecule. In the vast majority (21 out of 30) of tumors with budding margins and morphological features of epithelial-mesenchymal transition (EMT), L1CAM was focally expressed in 14/21 cases, and 3 out of 21 cases showed high L1CAM immunoreactivity. These properties of L1CAM suggest a major role for L1CAM in tumor cell migration in CRC and make it a potentially useful new marker for cancer progression and a candidate for anti-cancer therapy.

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Impact statement: This work highlights different expressions of L1CAM and their relative frequencies in colorectal cancer and provides new data regarding the interindividual variability in L1CAM expression in this tumor. L1CAM is here indicated as a marker associated with EMT. It is also hypothesized to may represent a new prognostic marker in CRC and a putative new anti-cancer target.

Abbreviations: L1CAM: L1 cell adhesion molecule; CRC: colorectal cancer; EMT: epithelial-mesenchymal transition; IgCAM: the superfamily of immunoglobulin-like adhesion molecules.

Key words: L1CAM; colorectal cancer; immunohistochemistry; grading; epithelial-mesenchymal transition.

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INTRODUCTION

The neural cell adhesion molecule L1 (L1CAM) is a stem cell marker belonging to the immunoglobulin superfamily of cell adhesion molecules (IgCAMs) (1). L1CAM is a 200-220 kDa transmembrane glycoprotein, with a long ectodomain that comprises six immunoglobulin-like (Ig) domains followed by five fibronectin type III repeats (2), a single transmembrane domain and a relatively short cytoplasmic domain (3). L1CAM is known for its role in neural development, being able to regulate processes such as neurite outgrowth, fasciculation, cell adhesion, cell migration, myelination, and cell survival, and allowing the construction of a dynamic neural network (4). L1CAM shows multiple functions, depending on its homophilic or heterophilic interactions with other L1CAM molecules (5, 6) or with cell-matrix components (7, 8), such as integrins, CD24, and other binding partners including neurocan or neuropilin-1 (5). Furthermore, the cytosolic tail of L1CAM interacts with several different binding partners, mediates interaction with the cytoskeleton proteins, and activates downstream signaling pathways (9, 10).

In physiology, L1CAM plays a fundamental role in fetal development and is highly expressed in the central (11) and peripheral nervous systems (12). Recently, L1CAM has been reported in multiple fetal organs, including the kidneys (13) and the gastrointestinal tract (14). However, L1CAM is aberrantly expressed in several types of human solid tumors, including endometrial cancer (15), ovarian cancer (16), and melanoma (17).

Previous studies have shown the association of L1CAM expression with metastasis in cancer (18). In particular, it has been hypothesized that L1CAM might represent a downstream target gene of the Wnt/ β -catenin signaling pathway, one of the most basic pathways involved in intercellular communications during development, that is aberrantly activated in colon cancer development and progression (19). Moreover, a study carried out in cells in culture, identified L1CAM as the most prominent E-selectin ligand, confirming a possible major role for this adhesion molecule in carcinogenesis (20). Recent studies on L1CAM expression in colorectal cancer reported that patients displaying high levels of L1CAM in tumor cells were characterized by a higher risk for metastasis (21). According to recent hypotheses, the enteric neuronal network should be involved in tumor cell migration in colon cancer (22, 23), partly via L1CAM expression. This

process, called perineural invasion, might occur along extrinsic nerves, with Schwann cells expressing L1CAM and providing physical guidance for tumor cells (24).

In recent times, L1CAM expression has been also reported in cancer-associated fibroblasts (CAFs) suggesting a further role for L1CAM in regulating cancer cell-stromal cell communications (25). Moreover, L1CAM has been identified as the most prominent ligand for E-selectin, a player in the binding of tumor cells to the endothelium, a major event in metastasis (26). These findings suggested a major role for L1CAM in the activity of tumor-associated stromal cells including the tumor-associated endothelial cells (TECs), cancer-associated fibroblasts (CAFs), and tumor-associated other inflammatory cells that produce growth factors, angiogenic factors, and proteolytic enzymes which might enhance tumor cell invasion and metastasis (27, 28). These findings allowed the establishment of a better link between L1CAM and metastasis (29).

Recently, the expression of L1CAM in colorectal cancer (CRC) has been indicated as an ancillary tool for stratifying carriers of colorectal cancer and identifying patients prone to dismal prognosis (30). The present study aimed to assess, using immunohistochemistry, the expression patterns of L1CAM in CRC, in order to correlate the immunohistochemical pattern of L1CAM with the aggressivity of tumor cells.

PATIENTS AND METHODS

We performed a retrospective study on formalin-fixed paraffin-embedded tissue samples (according to conventional techniques) of CRC, to test the immunoreactivity of tumor cells for L1CAM. The biopsy samples and tissue resections of CRC were obtained at the Division of Pathology of the University Hospital Agency of Cagliari.

In the present study, 51 patients affected by colorectal adenocarcinoma, ranging in age from 38 to 88 years, 21 females and 30 males, were analyzed. The main 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)

Table 1. Clinical, histological, and immunohistochemical data regarding 51 cases of colorectal cancer.

CASE	AGE	GENDER	TUMOR GRADE	BUDDING MARGINS	L1CAM SCORE	CDX2	MLH-1	PMS-2	MSH-2	MSH-6
1	66	F	G1	-	0	+	+	+	+	+
2	59	M	G2	-	0	+	+	+	+	+
3	61	F	G3	-	0	+	+	+	+	+
4	61	F	G3	-	0	+	+	+	+	+
5	65	M	G2	-	0	+	+	+	+	+
6	65	M	G2	-	0	+	+	+	+	+
7	51	F	G1	-	1	+	+	+	+	+
8	43	F	G1	-	1	+	+	+	+	+
9	67	F	G2	+	1	+	+	+	+	+
10	67	M	G3	-	0	+	+	+	+	-
11	81	M	G3	+	1	+	+	+	+	+
12	83	F	G2	+	2	+	+	+	+	+
13	82	F	G3	-	0	-	-	-	+	+
14	81	F	G2	-	0	+	-	-	+	+
15	66	F	G2	++	2	+	+	+	+	+
16	82	M	G2	-	1	+	+	+	+	+
17	88	F	G2	-	0	+	+	+	+	+
18	80	M	G1	+	1	+	+	+	+	+
19	79	M	G3	-	0	+	-	-	+	+
20	49	M	G2	+	1	+	+	+	+	+
21	49	M	G2	+	3	+	+	+	+	+
22	71	M	G3	-	0	+	+	+	+	+
23	59	M	G1	-	1	+	+	+	+	+
24	38	F	G3	+	1	+	+	+	+	+
25	70	M	G1	+	1	+	+	+	+	+
26	79	F	G2	+	1	+	+	+	+	+
27	75	F	G2	-	0	+	-	-	+	+
28	55	F	G1	-	1	+	+	+	+	+
29	49	F	G2	-	1	+	+	+	+	+
30	70	M	G2	-	1	+	+	+	+	+
31	74	M	G2	-	2	+	+	+	+	+
32	84	F	G2	+	1	+	+	+	+	+
33	47	M	G2	-	0	+	+	+	-	-
34	65	M	G2	+	3	+	+	+	+	+
35	81	M	G2	-	0	+	+	+	+	+
36	40	F	G2	+	3	+	+	+	+	+
37	84	M	G1	-	0	+	+	+	+	+
38	82	F	G2	-	0	+	-	-	+	+
39	67	M	G2	+	1	+	+	+	+	+
40	78	M	G2	+	1	+	+	+	+	+
41	70	M	G2	+	1	+	+	+	+	+
42	77	M	G1	-	0	+	+	+	+	+
43	85	M	G2	-	1	+	+	+	+	+
44	81	F	G2	-	0	+	+	+	+	+
45	54	M	G1	-	1	+	+	+	+	+

CASE	AGE	GENDER	TUMOR GRADE	BUDDING MARGINS	L1CAM SCORE	CDX2	MLH-1	PMS-2	MSH-2	MSH-6
46	58	M	G2	-	0	+	-	-	+	+
47	74	M	G2	+	2	+	+	+	+	+
48	74	F	G2	+	2	+	+	+	+	+
49	69	M	G2	+	2	+	+	+	+	+
50	78	M	G2	+	1	+	+	+	+	+
51	65	M	G2	+	1	+	+	+	+	+

BM: Budding Margins; CDX2: Caudal related homeobox gene; MLH1: mutL homolog 1; MSH2: mutS homolog 2; MSH6: mutS homolog 6; PMS2: homolog 2, mismatch repair system component; L1CAM: cell adhesion molecule L1.

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 semi-quantitatively by two independent Authors (Cau F and Faa G), 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 tumor cells. We regarded the staining as positive in cases with cytoplasmic, Golgian, and/or cell membrane positivity. No nuclear reactivity for L1CAM in tumor cells was detected. In cases of discrepant assessments, slides were reinvestigated by both Authors under a multi-head microscope and an agreement was obtained. The χ^2 test was used to examine the association between L1CAM expression and various clinicopathological characteristics, including age, gender, tumor location, and degree of differentiation. To obtain a semiquantitative evaluation of the degree of immunoreactivity for L1CAM, the following semiquantitative scoring system was applied: 0 = no reactivity; 1+ = <10% of immunoreactive tumor

cells; 2++ = 10-50% of immunoreactive cells; 3+++ = >50% of tumor cells immunostained for L1CAM (**Table 2**) (**Figure 1**). In addition, immunohistochemistry for CDX2 and for genes involved in microsatellite instability (MSI) (MLH-1, PMS-2, MSH-2, MSH-6) was performed, in order to investigate a possible correlation between L1CAM expression and MSI. 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 30 males and 21 female patients ranging from 38 to 88 years of age (mean, 68 years) who underwent surgery for colorectal cancer. Pertinent clinical, histological, and immunohistochemical findings regarding L1CAM expression in colorectal cancer are presented in **Table 1**. At histology, 10 tumors were well differentiated and were classified as slow-grade adenocarcinomas (G1), 22 showed a moderate grade of differentiation (G2) and the remaining 8 cases were scarcely differentiated and were classified as high-grade adenocarcinomas (G3). In 21 out of 51 cases, we observed budding margins, with tumor cells detaching from the tumor mass and infiltrat-

Table 2. The scoring system applied to the evaluation of immunostaining for L1CAM.

0	No reactivity of L1CAM
1+	<10% of immunoreactivity cells in membrane and cytoplasm
2+	>10% and <50% of immunoreactivity in membrane and cytoplasm
3+	>50% of cells immunostained in membrane and cytoplasm

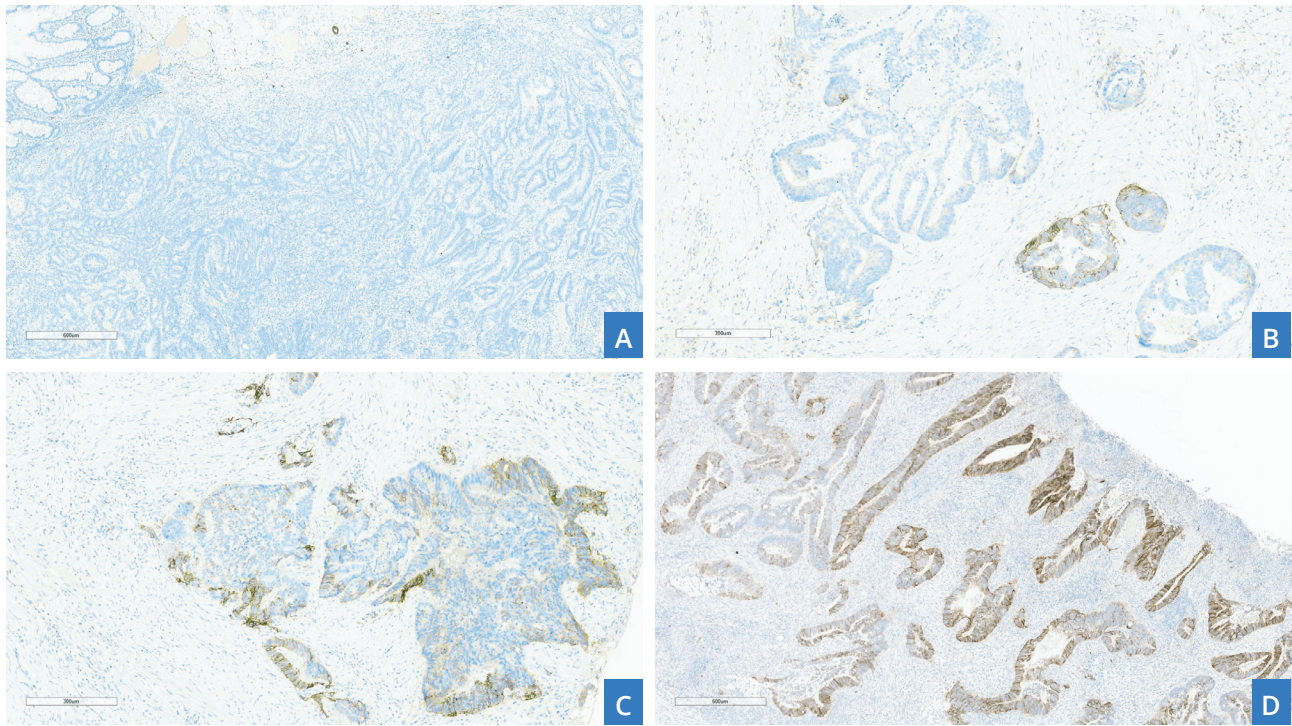


Figure 1. Scoring system for L1CAM. (A) No reactivity for L1CAM (score 0); (B) <10% of immunoreactive tumor cells (score 1); (C) 10% - <50% of immunoreactivity in tumor cells (score 2); (D) >50% of tumor cells immunostained for L1CAM (score 3).

ing the peritumoral microenvironment. In 50 out of 51 cases, tumor cells were immunoreactive for CDX2. Immunohistochemistry for the 4 genes involved in MSI revealed negative immunostaining for MLH-1 and PMS-2 in 6 out of 51 cases. All these 6 cases were also negative for L1CAM (**Table 1**). Immunohistochemical analyses regarding L1CAM expression in tumor cells showed immunostaining for L1CAM in 31 out of 51 cases (61%). No reactivity was detected in tumor cells in the remaining 21 tumors (39%). In positive cases, L1CAM was mainly expressed along the cell membrane, with some tumor cells expressing the adhesion molecule even in the cytoplasm. No nuclear reactivity was detected in this study in cancer cells.

A marked interindividual variability was observed regarding the degree of immunoreactivity for L1CAM in the 31 positive cases (**Table 1**): focal staining, involving less than 10% of tumor cells (score 1) was observed in 22 out of 31 positive cases (**Figure 1B**); in 6 cases, immunoreactivity was found in less than 50% of tumor cells (score 2) (**Figure 1C**); in the remaining 3 positive cases, a diffuse reactivity for L1CAM (score 3) was observed (**Figure 1D**).

A strict association was found between the presence of budding margins and the expression of L1CAM. This adhesion molecule was expressed in all 21 adenocarcinomas characterized by infiltrative margins

(see **Table 1**). All 21 colorectal cancers with budding margins were characterized by L1CAM expression, with a score of 3 in three cases, a score of 2 in 6 cases, and a score of 1 in the remaining 12 cases (**Table 1**). In tumors with budding margins, L1CAM was mainly expressed in tumor cells acquiring an invasive and motile phenotype, detaching from the tumor mass, and infiltrating the peritumoral microenvironment (**Figure 2A, B**). In tumor cells with an infiltrative phenotype and with morphological features suggestive of epithelial-to-mesenchymal transition, L1CAM was strongly expressed along the cell membrane. The strong immunostaining for L1CAM observed in infiltrating cancer cells often contrasted with the absence of expression in the tumor mass (**Figure 3**). No significant correlation was observed between the degree of differentiation of colorectal cancer cells and L1CAM expression in tumor cells. Cases with the highest scores for L1CAM expression, score 2 and score 3, were all classified as intermediate grade of differentiation (G2) (see **Table 1**). L1CAM expression was unevenly distributed in the tumor mass. Areas with strong reactivity for L1CAM were frequently observed adjacent to areas negative for the cell adhesion molecule (**Figure 4**). L1CAM expression was not restricted to tumor cells, being detected also in other components of the tumor microenvironment. L1CAM was occasionally ob-

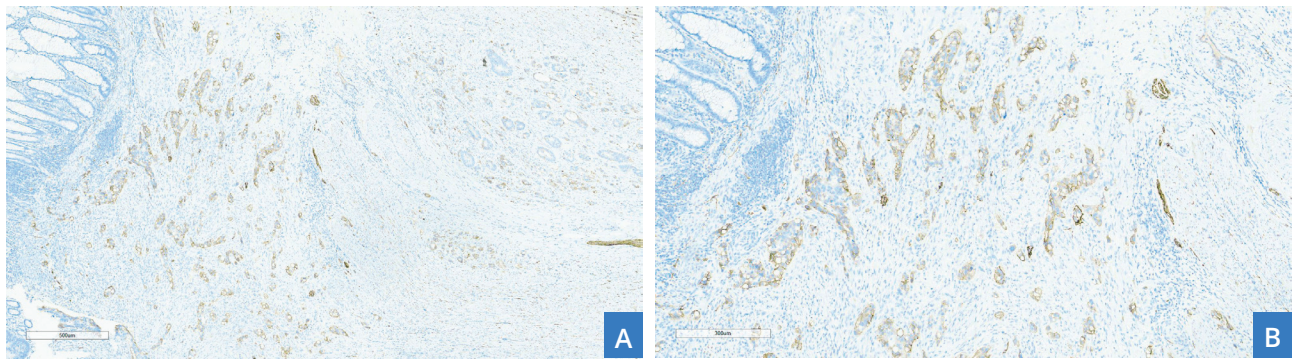


Figure 2. (A) At low power, tumor cells acquiring a motile phenotype show strong reactivity for L1CAM; (B) at higher power, immunostaining for L1CAM appears mainly localized at the cell membrane of cancer cells.

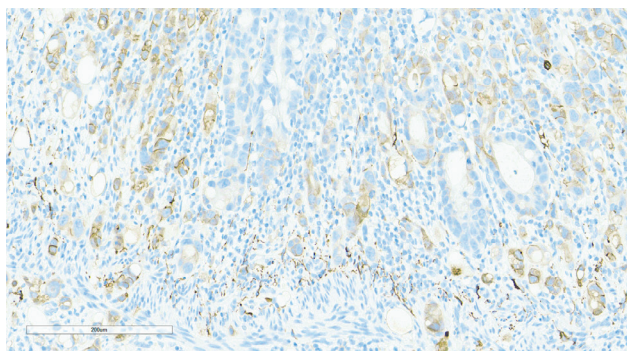


Figure 3. Strong reactivity for L1CAM in tumor cells infiltrating the peritumoral environment contrasts with the absence of immunostaining in the tumor mass.

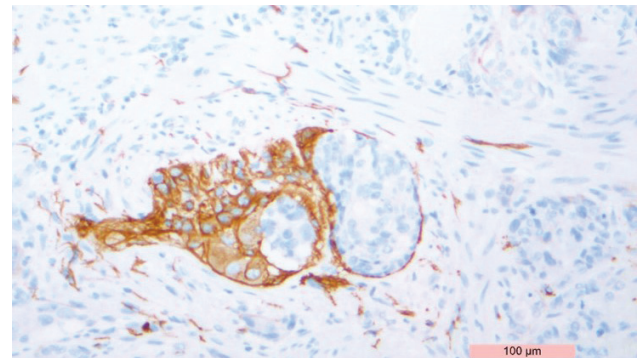


Figure 6. The strong reactivity of nervous structures for L1CAM allows better identification of perineural invasion of cancer cells, with L1CAM-positive Schwann cells providing physical guidance for tumor cells.

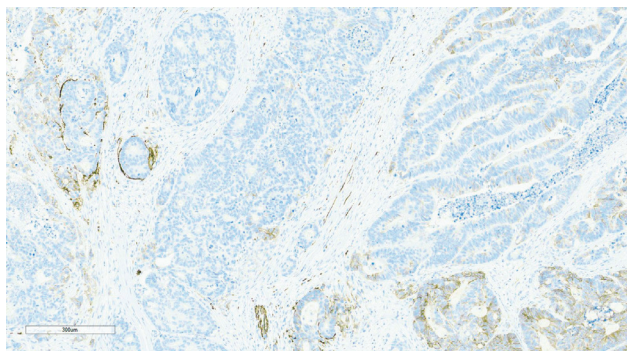


Figure 4. This picture shows cancer cells with strong immunostaining for L1CAM adjacent to negative tumor cells.

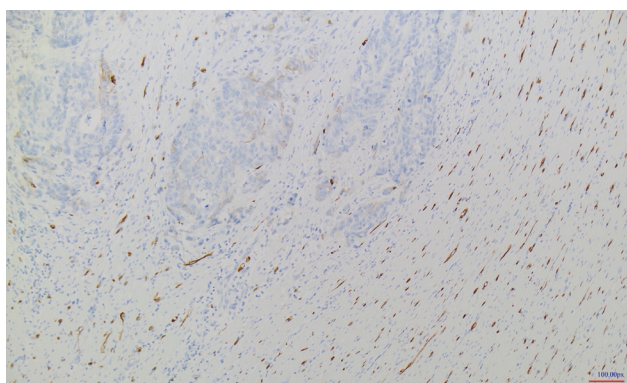


Figure 5. L1CAM expression in cancer-associated fibroblasts.

served in stromal cells surrounding the tumor mass, including cancer-associated fibroblasts (CAFs). In these cells, L1CAM was expressed along the cell membrane and inside the cytoplasm (**Figure 5**). Moreover, L1CAM was strongly expressed in the nerve structures and in the ganglia cells of the intestinal wall. The strong reactivity of nervous structures for L1CAM allowed a better identification of the process called perineural invasion occurring along extrinsic nerves, with Schwann cells expressing L1CAM and providing physical guidance for tumor cells (**Figure 6**).

Among the 51 cases of colorectal adenocarcinomas analyzed, 8 cases were already treated with neoadjuvant therapy, 3 of which were negative for L1CAM in tumor cells. In the 5 remaining cases, in 3 the expression for L1CAM was focal (score 1), and in 2 cases L1CAM expression was detected in 10%-50% of tumor cells (score 2).

The χ^2 statistical analysis did not highlight any significant association between L1CAM expression and the other variables analyzed in this study, both using the four classes of L1CAM expression (χ^2 (6, N = 51) = 10.4, p = .108) and the two classes approach (χ^2 (2, N = 51) = 5.96, p = .051).

DISCUSSION

CRC is currently the third leading cause of cancer death worldwide. Among the main risk factors for the disease, in addition to age, an unvaried and unbalanced diet, smoking, and random errors in the DNA occurring during cell division may be included (31).

The most common colon-rectal tumors are represented by adenocarcinomas (95%), followed by less frequent forms such as carcinoids, gastrointestinal stromal tumors, lymphomas, squamous cell carcinomas, leiomyosarcomas and melanomas (32, 33). A majority of cases of CRC develop sporadically, without a family history or hereditary genetic predispositions (34) causing uncontrolled cell growth of tumor cells themselves. The efficacy of the treatments available for the treatment of colorectal cancer has improved significantly in recent years, thanks to the identification of specific molecular targets which could improve and make more decisive the most suitable treatments for each patient (35).

In this study, we analyzed L1CAM expression in tumor cells in a large series of CRC. The most important finding emerging from our work is the marked interindividual variability of L1CAM immunoreactivity in colon cancer cells, with 31 out of 51 (61%) cases expressing L1CAM. In positive cases, the expression of L1CAM in tumor cells ranged from mild immunostaining in 22 cases (score 1), to moderate reactivity in 6 (score 2), strong and diffuse expression (score 3) being restricted to 3 tumors (see **Table 1**). This is a new finding regarding L1CAM expression in CRC, which evidences that in a large cohort of CRCs (39%) tumor cells are not immunoreactive for this cell adhesion molecule. Moreover, in 22 out of 30 positive cases, immunoreactivity was mild and focal, L1CAM being expressed in less than 10% of tumor cells. Grouping the negative cases (score 0) (**Figure 1A**) with cases with low reactivity (score 1) (**Figure 1B**), the vast majority (42/51) of CRCs were characterized by the absence or by a very low expression of L1CAM. Grouping cases with moderate immunostaining (score 2) and cases characterized by a strong expression (score 3), only 9 out of 51 CRCs analyzed in this study (18%) showed a remarkable expression of L1CAM.

In positive cases, a marked intratumoral variability among tumor cells was also observed, regarding the expression of L1CAM. Cancer cells with strong immunostaining for L1CAM were frequently observed in strict contact with negative cells (**Figure 4**). Moreover, in some tumors, the front invasion of the tumor was characterized by higher

levels of L1CAM expression as compared to the superficial areas of the tumor mass. In CRC cases characterized by budding margins, it was possible to observe a strong expression of L1CAM in cells acquiring a motile and infiltrative phenotype with morphological features suggestive for epithelial-to-mesenchymal transition (EMT). The high expression of L1CAM in infiltrating cells often contrasted with the absence or low reactivity for L1CAM in the adjacent tumor cells (**Figure 3**).

Focusing on the 9 cases with medium-high L1CAM expression, their score ranging from 2 to 3, all cases were characterized by budding margins with histological evidence of tumor cells detaching from the tumor mass. These findings reinforce the hypothesis of a strong association between high levels of L1CAM expression and a motile phenotype in cancer cells.

In previous studies, it has been suggested that L1CAM might play a dualistic role: i) a static function, acting as an intercellular adhesion molecule that functions as a glue between cells forming zipper-like structures along the intercellular boundaries (36); ii) a motility-promoting function, driving cell migration during development and allowing tumor cell invasion (37). According to this hypothesis, the switch in the functional mode of L1CAM might be triggered by the following factors: i) the cleavage from the cell surface of the long ectodomain of L1CAM by membrane proximal proteolysis (38); ii) the ability to change binding partners, moving from the homophilic binding typical of the static function to the heterophilic bindings, including integrin binding, in the motility-promoting mode (2, 39). By augmenting its interactions with Beta-1-integrins, L1CAM might drive cell migration both in physiology and in pathological settings (40). In oncology, L1CAM has been previously reported to be overexpressed in multiple cancers, including ovarian cancer, endometrial cancer, pancreatic adenocarcinoma, melanoma, and glioblastoma (41, 42). In the tumor setting, L1CAM has been associated with the induction of an invasive and motile phenotype in tumor cells, supporting an aggressive clinical behavior and favoring vascular invasion and metastases (43). In experiments carried out on colon cancer cells *in vitro*, L1CAM was able to increase cell motility and growth, whereas its suppression in colon cancer cells decreased motility (44). Moreover, the up-regulation of L1CAM has been associated with the up-regulation of

epithelial-mesenchymal transition (9-45). On one hand, our findings confirm these previous studies on the relevance of L1CAM in favoring an invasive and motile phenotype in tumor cells in colon cancer (41). In 9 out of 9 cases characterized by medium-high expression of L1CAM in tumor cells, L1CAM expression was higher in tumors with budding margins, and in cells showing an infiltrative phenotype as compared to tumor cells of the tumor mass (**Figure 3**).

Moreover, in cases with a low L1CAM score, we occasionally observed tumor cells with invasive behavior negative for L1CAM. The interpretation of these findings appears complex and evidences that the molecular bases of invasion in colorectal cancer have not been completely elucidated yet. We may hypothesize that different molecular pathways might be involved in the induction of invasion and metastasis in CRC. On one hand, our findings confirm the existence of an L1CAM-dependent signaling pathway in which L1CAM promotes a motile phenotype in cancer cells (9, 46). In this pathway, up-regulation of L1CAM might be induced by TGF-Beta-1 (47). On the other hand, the finding of fields in which the acquisition of a motile phenotype in tumor cells was associated with the absence of immunoreactivity for L1CAM, clearly indicates that other molecules and other molecular pathways should be involved in tumor cell invasion.

In previous studies from our group, Thymosin Beta-4 was demonstrated at the invasion front of CRC, being mainly expressed in tumor cells undergoing EMT (48). The immunohistochemical data were subsequently confirmed by a proteomic study (49). The expression of TB4 might represent an alternative molecular pathway involved in EMT in colon cancer and might explain the absence of L1CAM found in a subset of tumor cells undergoing EMT.

In this study, as highlighted by the statistical analysis, we have not observed any significant association between L1CAM expression and the histological tumor grade. Among the 8 cancers with G3 grading, 6 were negative for L1CAM whereas the remaining 2 cases showed a mild expression (score 1) of the cell adhesion molecule. These findings indicate that L1CAM might play different roles in cancer insurgence and progression and that L1CAM expression is not related to the degree of differentiation of cancer cells.

The absence of L1CAM expression in 6 cases characterized by negative immunostaining for two

genes involved in microsatellite instability, MLH-1, and PMS-2, remains, to the best of our knowledge, unexplained and deserves further studies on a larger series of colorectal cancers. Nevertheless, there could still be a weak or moderate association between the variables that was not detected due to limitations in the sample size or measurement methods. Therefore, further research may be needed to confirm these conclusions and explore any potential practical implications of the reported results.

A recent study on L1CAM expression in ovarian cancer suggested the hypothesis that L1CAM might induce stemness in cancer cells, indicating that this adhesion molecule could be involved in cancer stem cell induction and regulation (16). This study is in agreement with a previous study from our group, which identified the stem cell marker CD44 in colon cancer cells, this molecule being associated with an unfavorable prognosis (50). Another interesting finding emerging from this study is the ability of L1CAM to evidence marked changes in the neurons of the local enteric nervous system (SEL). The expression of L1CAM was present in tumor cells spreading along the nerve fibers. This finding may indicate the involvement of the peritumoral SEL which could promote, through specific receptors, the proliferation of tumor cells by the expression of pro-survival signaling pathways (22).

All these data taken together, L1CAM appears as a fascinating molecule with multiple functions both in physiology and pathology. Given the role played by L1CAM in the induction of a motile and infiltrative phenotype in tumor cells, we suggest its introduction in clinical practice, in order to better characterize all CRCs. Further studies should be carried out to better evidence the linkage between L1CAM expression and the clinical behavior of patients affected by CRC. These properties of L1CAM, in addition to its cell surface localization, make it a potentially useful diagnostic marker for cancer progression and a candidate for personalized anti-cancer therapy.

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COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of interests

The Authors have declared no conflict of interests.

Availability of data and materials

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

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.

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.

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