

Article

Preliminary study on the mechanism of L1CAM promoting invasion and metastasis of cervical cancer

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Abstract: Objective: To explore the presentational characteristics of cell adhesion molecule L1 gene (L1CAM) in cervical cancer and its bidirectional regulation of tumor cell proliferation, migration, invasion and apoptosis, and to address the possible psychosocial problem of L1CAM-tumor aggressiveness to the psychological well-being and quality of life and psychosocial adaptation of patients. **Methods:** The mRNA expression level of L1CAM was detected in cervical cancer cell lines (HeLa, SiHa, C-33A) and normal cervical epithelial cells (HcerEpic) using Quantitative Polymerase Chain Reaction (qPCR), and L1CAM expression was regulated using siRNA knockdown and plasmid overexpression techniques. Combined with Western Blot (WB) to verify the efficiency of gene manipulation, CCK-8 assay was used to scout cell proliferation viability, Transwell assay to assess migration and invasion ability, and flow cytometry to analyze the apoptosis rate. The variables of psycho-oncological variables, such as psychological distress and anxiety, depression, fear of recurrence, and quality of life, which are measured using validated tools, like the HADS, PHQ-9, GAD-7, and EORTC QLQ-C30, are theoretically incorporated into the discussion and the way L1CAM expression can be correlated with these patient-centered outcomes is proposed. **Results:** L1CAM had a relatively higher expression level in the Hela, C-33A than other cervical cell lines. After knocking down L1CAM by siRNA, the proliferative, migratory and invasive capacities of cervical cancer cells were significantly reduced, the apoptosis rate was markedly increased. Conversely, overexpression of L1CAM significantly enhanced cellular proliferation, migration and invasion while suppressing apoptosis. **Conclusion:** L1CAM is aberrantly expressed in cervical cancer and has the potential for bi-directional regulation of oncogenic processes, which promotes tumor progression by enhancing proliferation, migration, invasion and inhibiting apoptosis, and targeting L1CAM may provide a new strategy for the treatment of cervical cancer. Higher tumor aggressiveness promoted by L1CAM overexpression at psychosocial level conceptually relates to poor prognosis, increased intensity of treatment, and increased emotional distress, implying that profiling of L1CAM expression patterns can identify patients at high psychosocial risk and allow early interventions to support them. The relationship between biomarker status, psychological burden, and the experience of survivorship should be directly correlated with the L1CAM expression in future clinical studies to clarify the association of L1CAM expression with validated psychosocial measures.

Keywords: cell adhesion molecule L1 gene; cervical cancer; tumor cell proliferation; migration; invasion; apoptosis; mRNA expression level; psycho-oncology; psychological well-being; quality of life; emotional distress; psychosocial adjustment; supportive care

1. Introduction

Cervical cancer is one of the most common malignant tumors among women

worldwide, posing a serious threat to women's health [1,2]. Without effective cervical cancer screening programs, many women in low-resource countries are diagnosed with invasive disease. Local invasion and distant metastasis significantly worsen patient survival outcomes [3]. Early-stage patients are mainly treated with surgery, and postoperative adjuvant therapy is selected according to high-risk factors, and the prognosis is generally better [4]. For advanced cervical cancer, treatment can involve surgery, chemotherapy, and/or radiotherapy, with external beam radiation therapy and brachytherapy as key components [3].

In addition to its biological and clinical importance, tumor aggressiveness in cervical cancer is not just a determinant of survival, but also a serious contributor to the psychological well-being of patients, emotional distress, quality of life (QoL), and even psychosocial adaptation. It is commonly observed that women diagnosed with cervical cancer have high anxiety, recurrence anxiety, and fears, sexual dysfunction and disturbed social role functioning, which are considerably increased in case of aggressive or advanced cervical cancer [3,4]. The psychosocial effects of being diagnosed with cervical cancer are also complicated by the fact that the condition itself has human papillomavirus (HPV) infection (that may induce stigma, guilt, and distress in relations). In the light of psycho-oncology, the determination of the molecular forces behind the aggressiveness of cervical cancer is thus a biological necessity, not to mention that it is a precondition to predicting and fulfilling the psychosocial demands of patients. To better define this relationship between biomarker status and psychologicTo enhance the psycho-oncology model, there is need to directly correlate L1CAM expression with validated psychosocial measures to clarify how biological tumor features, like L1CAM-driven aggressiveness, can directly affect psychological adaptation, the survivorship experience, and psychosocial care requirements. The validated instruments used to measure these patient-centered outcomes in oncology studies are standardized measures such as the Hospital Anxiety and Depression Scale (HADS), Patient Health Questionnaire-9 (PHQ-9), Generalized Anxiety Disorder-7 (GAD-7) and EORTC Quality of Life Questionnaire (QLQ-C30). It is necessary to explore the process of cervical cancer occurrence and development, and further reveal its specific molecular mechanisms. It has been shown that relevant proteins regulating cell adhesion, cytoskeleton and cell migration are high-risk factors, which play a key role in the invasion and metastasis of tumor cells [5–7].

Relevant studies have found that L1CAM is abnormally expressed in patients with cervical adenocarcinomas, and the expression level of L1CAM is closely related to the aggressiveness of cervical cancer and the prognosis of patients [8,9]. β -catenin/TCF pathway promotes the expression of L1CAM, and directly up-regulates the transcriptional activity of L1CAM through the transcription factor Slug [10–12]. In addition, the p53 oncogene can promote ubiquitination degradation of Slug by forming a WTp53-MDM2-Slug complex with MDM2. And p21-activated kinase 4 (PAK4) can directly phosphorylate Slug, leading to Slug stabilization and enhancement of the EMT process, which in turn promotes tumor invasion and metastasis [13,14]. These studies suggest that L1CAM may be one of the important molecules in the invasion and metastasis of cervical cancer, and that PAK4 can promote the progression of cervical cancer by activating the Slug/L1CAM pathway in the presence of abnormal p53 function.

At present, studies on the role and regulatory mechanism of L1CAM in cervical cancer invasion and metastasis are still limited. In this paper, we dissected the expression characteristics of L1CAM in cervical cancer cells. Then we explored the effects of modulating L1CAM expression on cervical cancer cells and found that

it significantly influenced cell proliferation, metastasis, and invasion. Considering this psychosocial and biological topicality, we also comment on the theoretical implications of these results on the psycho-oncological care.

1.1. Psychosocial context of cervical cancer: A theoretical framework

The intensity of the cancer such as the cancer stage, invasiveness, and likelihood of metastasis has always been identified as a major determinant of psychological morbidity by psycho-oncology research. Biopsychosocial model of cancer care is an approach which assumes that the biological tumor properties and the psychological and social factors interplay to determine patient outcomes, such as treatment adherence, quality of life, and survival [14]. Psychosocial distress is common in cervical cancer in particular: it has been reported that 30-50 percent of patients develop clinically significant anxiety or depression with the highest rate of prevalence among patients with advanced disease, distant metastasis or recurrence [15]. Several validated screening instruments, including the Distress Thermometer, HADS, PHQ-9, and GAD-7, are regularly employed to measure these symptoms, which provide the opportunity to identify patients who could receive a psycho-oncological intervention.

New studies in psychoneuroimmunology and neuroendocrine oncology further indicate that it is possible that psychological stress is not simply a reflection of tumor aggressiveness, but it indirectly controls tumor biology. Chronic psychological stress triggers hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system, which results in an increase in the amount of glucocorticoids and catecholamines in the circulation. Such stress hormones have been demonstrated to induce the release of pro-inflammatory cytokines, inhibit natural killer (NK) cell functions, and initiate signaling pathways—e.g., MAPK/ERK and PI3K/AKT—which overlap with those involved in cancer cell survival, proliferation, and invasion [15,16]. Though it is not directly the measurement of the psychological variables or neuroendocrine mediators used in the in vitro experimental design of this study, this theoretical framework puts L1CAM in a context of a wider psychobiological environment and encourages future applications of this study at the molecular oncology/psycho-oncology interface. Further clinical studies are needed to directly investigate the relationship between L1CAM levels of expression with psychosocial outcomes that will be gathered in the future, such as psychological distress, anxiety, depression, fear of recurrence, coping mechanisms, and quality of life, and how the concept of disease aggressiveness and awareness of prognosis contributes to emotional burden.

2. Materials and methods

2.1. Cell modeling and culture

Here are the cell line sources:

- 1) Tumor cell lines: HeLa, SiHa, C-33A (Bei Na Bio).
 - 2) Normal control: HcerEpic human cervical epithelial cells (Huatuo biology).
- Culture conditions: Cultured cells in DMEM medium containing 10% high-quality fetal bovine serum and 1% penicillin-streptomycin double antibody, with 1 change of fluid in 2–3 d. Cells were grown adherent to the wall, which could be carried out according to the ratio of 1:3 passaging, and were cultured at 37 °C, 5% CO₂ and saturated humidity in an incubator.

2.2. Experimental design

2.2.1. L1CAM expression profile analysis

Human cervical cancer cell lines (Hela, SiHa, C33A) and normal cervical

epithelial cells (HcerEpic), 3 biological replicates per group, 12 samples in total, numbered A-1 to D-3. Cells were lysed with RNAiso Plus, the aqueous phase was taken after chloroform fractionation and the RNA deposited by isopropanol. Then they were washed with 75% ethanol, and dissolved in RNase-free water [15].

Using Hifair® III kit, RNA was digested by gDNA and random primers and reverse transcriptase were added 55 °C for 15 min to generate cDNA.

The qPCR primer sequences were designed as displayed in **Table 1**, and the reaction was carried out in 10 µL containing 5 µL of SYBR Green Master Mix, 0.4 µM of each primer, and 1 µL of cDNA template, and the amplification procedure was, pre-denaturation at 95 °C for 2 min. 40 cycles of 10 s at 95 °C, and 30 s at 60 °C, and melting curve was analyzed at 60 °C–95 °C .

Table 1. qPCR primer sequence design.

Gene name	Nucleic acid sequence	Amplification length (bp)
L1CAM-F	AGGAAGGGGAGTCAGTGGTT	173
L1CAM-R	GTCTGAGTGGTTGTCGGAGG	
GAPDH(homo)-F	AGATCCCTCCAAAATCAAGTGG	130
GAPDH(homo)-R	GGCAGAGATGAIGACCCTTTT	

2.2.2. Genetic manipulation

L1CAM gene silencing was performed on HeLa cells using the Lipofectamine2000 transfection system, and the siRNA sequences targeting L1CAM are shown in **Table 2**. HeLa cells were first inoculated at 60% density in 6-well plates containing 10% FBS and 1% double antibody in DMEM medium. Succeedingly, the original medium was abandoned after the cells were attached to the wall, washed twice with PBS, and finally the transfection complexes were prepared in an ultra-clean bench 100 pmol siRNA with 5 µL Lipofectamine2000 per well was diluted with 250 µL Opti-MEM, respectively, and the liposome-siRNA complexes were mixed after standing at room temperature for 5 min, and incubation was continued for 20 min, and the complexes were added drop by drop to the cells that had been replaced with serum-free culture medium. After 6 h of incubation at 37 °C and 5% CO₂, the medium was changed with complete medium, and the knockdown efficiency of siRNA on L1CAM was verified by qPCR after 48 h of transfection.

Table 2. siRNA sequences targeting L1CAM.

Primer name	Sequence (5'-3')	Length	MW(g/mole)
L1CAM-siRNA1	GCAAGAUCUUGCACAUCAAGC	21	6679.11
	UUGAUGUGCAAGAUCUUGCUG	21	6691.04
L1CAM-siRNA2	CAUCUACCGCUGCUUUGCCAGCAAU	25	7854.74
	AUUGCUGGCAAAGCAGCGGUAGAUG	25	8100.97
L1CAM-siRNA3	CCAAGUGGCCAAAGGAGACAGUGAA	25	8130.06
	UUCACUGUCUCCUUUGGCCACUUGG	25	7825.65
L1CAM-siRNA-NC	UUCUCCGAACGUGUCACGUTT	21	6583.01
	ACGUGACACGUUCGGAGAATT	21	6732.19

The relative expression of L1CAM was calculated by qPCR, and the siRNA sequence with the highest inhibition rate was screened using the siRNA-NC group as a control.

Figure 1 shows the experimental flow of overexpression plasmid construction, constructing L1CAM overexpression plasmid and transfecting cells by liposome method.

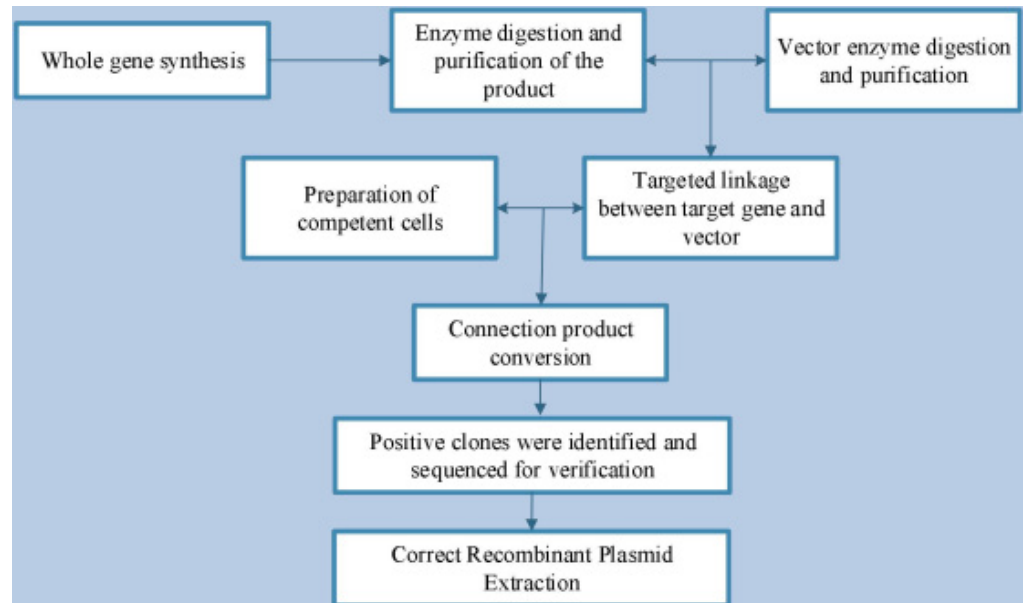


Figure 1. Experimental process of overexpression plasmid construction.

Using pCDH-CMV-MCS-EF1-copGFP-puro as the expression vector, the pCDH-CMV-MCS-EF1-copGFP-puro vector mapping information is shown in **Figure 2A**. The L1CAM gene sequence (NCBI Gene ID: 3897) was inserted by XbaI (TCTAGA) and EcoRI (GAATTC) double cleavage sites. Mix 10 µL of plasmid with 1 µL each of QuickCut™ XbaI/EcoRI in 1 × QuickCut Buffer and react at 37 °C for 1 h. Recover the linearized vector. The synthesized sticky end- containing L1CAM fragment (TCTAGA.... ..GAATTC) was mixed with the enzymatic vector at a 5:1 molar ratio, T4 ligase was added, and ligation was performed at 16 °C for 30 min. Transformed the ligation product into DH5 α receptor cells, which were ice-bathed for 30 min → heat-excited at 42 °C for 90 s → resuscitated at 37 °C for 45 min, coated on LB plates containing 50 µg/mL ampicillin, and cultured at 37 °C for 16 h before singling out the monoclonal clones. Correct cloning was confirmed by XbaI/EcoRI double digestion and sequencing. Correct cloning was confirmed by XbaI/EcoRI double digestion and sequencing (primer: T7 promoter/terminator), and high purity plasmids (OD260/280 = 1.8–2.0) were prepared using the Plasmid Bulk Extraction Kit (Omega)., and high purity plasmids (OD260/280 = 1.8–2.0) were prepared using the Plasmid Bulk Extraction Kit (Omega). HeLa cells were inoculated in 6-well plates at a density of 50%–60% and cultured using DMEM medium containing 10% fetal bovine serum and 1% double antibody. 4 µg of either L1CAM-pCDH plasmid or empty pCDH control plasmid was diluted in 250 µL of Opti-MEM serum-free medium, while 10 µL of Lipofectamine 2000 was separately diluted in another 250 µL of Opti-MEM. After 5 min of incubation at room temperature, the diluted plasmid and Lipofectamine solutions were combined and mixed gently, followed by a 20-min incubation at room temperature to allow DNA-lipid complex formation. The original medium was discarded, and the transfection complex was added (hatched at 37 °C,

5% CO₂ for 6 h) and replaced with complete medium to continue incubation for 48 h. The expression of copGFP was observed under fluorescence microscope, as shown in **Figure 2B**. The L1CAM protein overexpression level was detected by WB.

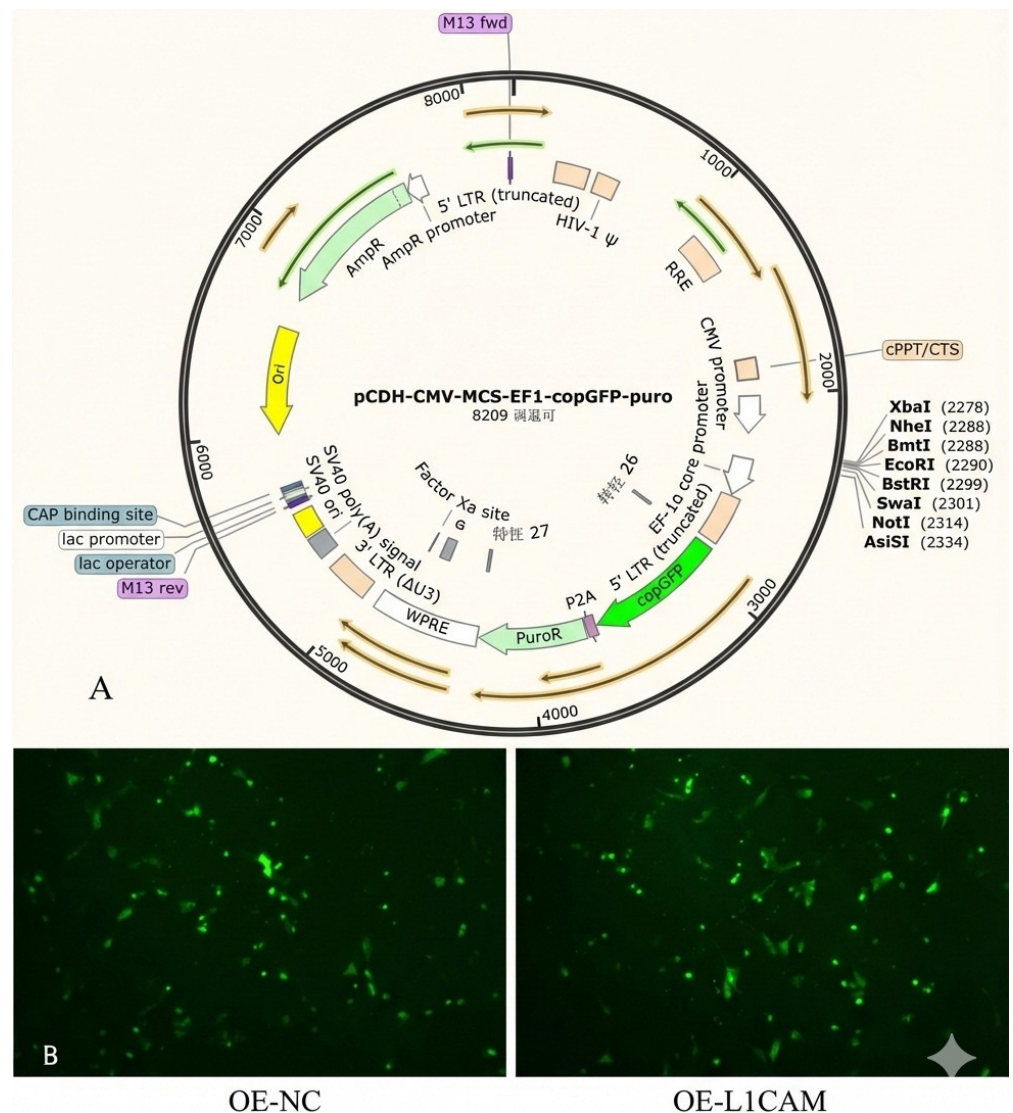


Figure 2. Vector image information and expression results under copGFP fluorescence. (A) pCDH-CMV-MCS-EF1-copGFP-puro vector map information; (B) The copGFP expression was observed by fluorescence microscopy. (OE-NC: empty plasmid transfection group. OE-L1CAM: overexpression L1CAM transfection group).

2.2.3. Functional validation

Functional validation included WB assay, cell proliferation, migration and invasion, and apoptosis analysis as follows:

1) HeLa cells were divided into 5 groups, which were blank control group (A1–A3), si-NC transfection group (B1–B3), si-L1CAM transfection group (C1–C3), empty plasmid transfection group (D1–D3), and overexpression L1CAM transfection group (E1–E3). Total protein was extracted using RIPA lysate, and the concentration was determined by BCA method. The final concentration of the samples was 2803.6–5106.7 µg/mL, and 20 µg of the samples were subjected to SDS-PAGE electrophoresis using 10% separator gel and 5% concentrate gel, and the membrane was closed after transferring to PVDF membrane (The membrane was washed with TBST for 1 min,

followed by blocking with 5% skimmed milk powder- TBST at room temperature for 1 h). Primary antibody incubation conditions were rabbit-derived L1CAM antibody (1:1000) and β -actin antibody (1:1000, overnight at 4 °C), and the secondary antibody was HRP-labeled goat anti-rabbit IgG, 1:1000, at room temperature for 1 h.

2) For cell proliferation, cells were inoculated in 96-well plates at 5×10^3 /well, added 10 μ L of CCK-8 reagent to each well after siRNA and plasmid were transfected respectively, and the absorbance at 450 nm was measured by an enzyme marker after hatch at 37 °C for 2 h.

3) Migration and aggression Experiments were excuted using Transwell chambers with 8- μ m pore size. DMEM containing 10% FBS was added to the lower chamber, and the upper chamber was inoculated with 2×10^5 /mL serum-free cell suspension and stained with crystal violet after 24 h. Pre-coated with Matrigel (1:3 dilution) for small chamber membranes, the remaining steps were similar as migration experiments.

4) Apoptosis analysis was excuted by Annexin V-PE/7-AAD double staining method, cells were collected 48 h after transfection, resuspended with Binding Buffer, incubated with 5 μ L of Annexin V-PE and 5 μ L of 7-AAD for 15 min under avoidance of light, and detected by flow cytometry (CytoFLEX).

2.3. Statistical analysis

The statistical analyses in this study were executed utilizing GraphPad Prism 10.0.

The data were represented as the mean and standard deviation, derived from a minimum of three independent experiments. Differences among groups were assessed by one-way analysis of variance (ANOVA), with a *p*-value < 0.05 considered statistically significant.

3. Results

3.1. Characterization of L1CAM expression in cervical cancer cells

The presentation levels of L1CAM mRNA in cervical cancer cell lines (HeLa, SiHa, C-33A), as well as normal cervical epithelial cells (HcerEpic), were detected by qPCR. The result was shown in **Figure 3**. qPCR revealed that L1CAM had a relatively higher expression level in the HeLa, C-33A than other cervical cell lines.

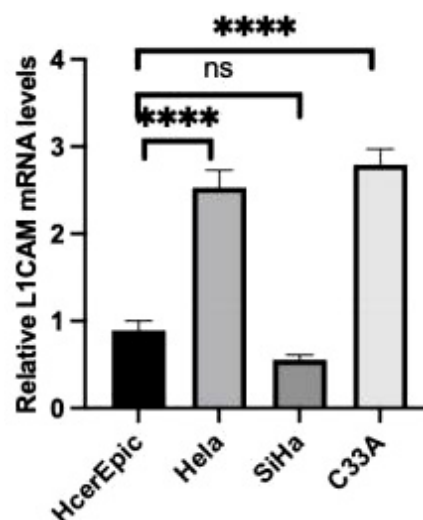


Figure 3. Indicates the expression level of L1CAM in cervical cell lines detected by qPCR.

**** indicates *P* < 0.0001. ns indicates that the difference is not statistically significant. The same below.

3.2. Validation of gene manipulation efficiency

L1CAM siRNAs induced down-regulation of L1CAM mRNA expression in HeLa cells. qPCR were used to assess L1CAM siRNA transfection efficacy and L1CAM mRNA expression. As shown in **Figure 4**, significant down-regulation of L1CAM mRNA expression was observed by transfection with three L1CAM siRNAs in HeLa cells as compared to the siRNA-NC group. Among the three siRNAs, L1CAM siRNA1 demonstrated the highest silencing efficiency in HeLa cells. Therefore, siRNA-1 was chosen for further experiments in this study.

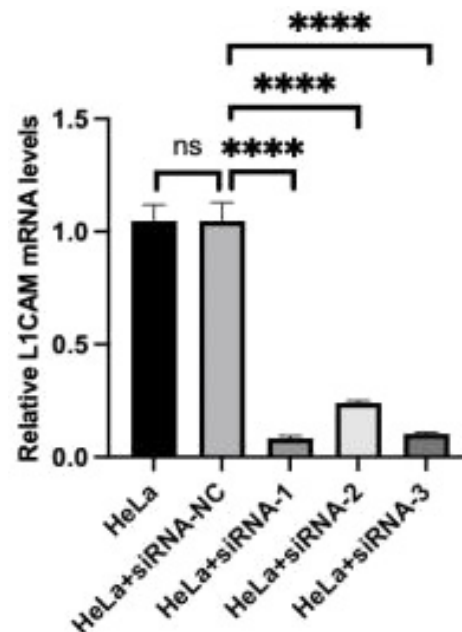


Figure 4. qPCR detection results of the relative L1CAM mRNA expression levels of siRNA in the three groups.

In terms of validation of siRNA knockdown efficiency, **Figure 5** shows siRNA transfection of group WB protein bands. A indicates the blank control group, B indicates the si-NC transfection group, and C is the si-L1CAM transfection group.

L1CAM protein expression was significantly down-regulated in the siRNA-transfected groups.

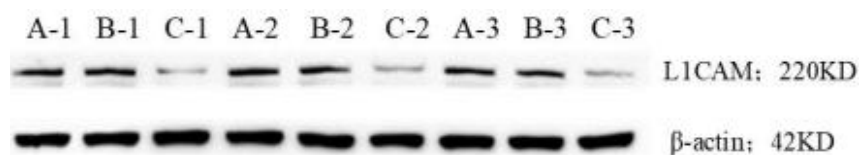


Figure 5. WB protein bands in siRNA transfection group.

The expression results of L1CAM verified by WB are shown in **Figure 6**. When knocking down L1CAM gene in the HeLa cell line, Western blot analysis revealed that compared with the blank control group, the si-L1CAM transfection group showed significantly reduced protein expression levels, while no significant difference was observed between the control group and the si-NC transfection group.

For the verification of overexpression efficiency, WB protein bands of overexpression groups are shown in **Figure 7**, A is the blank control group, D denotes the empty plasmid transfection group, and E represents overexpression L1CAM transfection group. It can be seen that in the L1CAM overexpression plasmid transfection groups (E-1, E-2, E-3), the L1CAM/β-actin ratio was significantly increased.

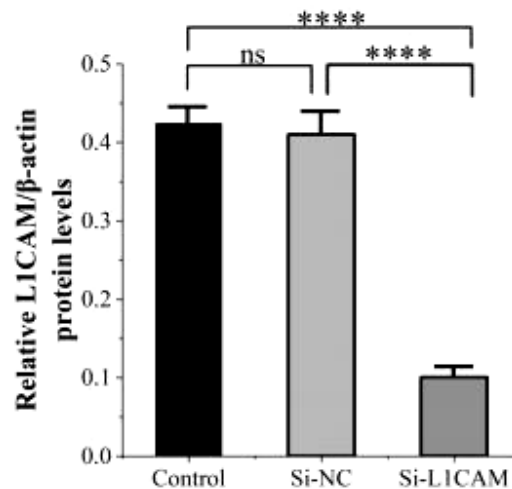


Figure 6. Expression results verified by WB when the L1CAM gene was silenced.

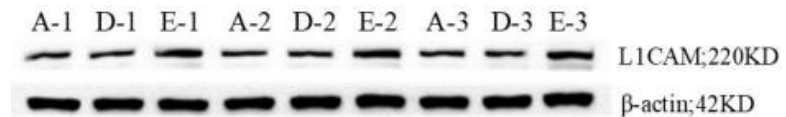


Figure 7. WB protein bands in overexpression group.

When overexpressed L1CAM gene in the Hela cell line, the expression results of L1CAM verified by WB are shown in **Figure 8**. WB revealed that the expression of L1CAM in the overexpression L1CAM transfection group was significantly increased in comparison with the control group, while no significant difference was observed between the control group and the empty plasmid transfection group. WB revealed that the expression of L1CAM in the overexpression L1CAM transfection group was significantly increased in comparison with the control group.

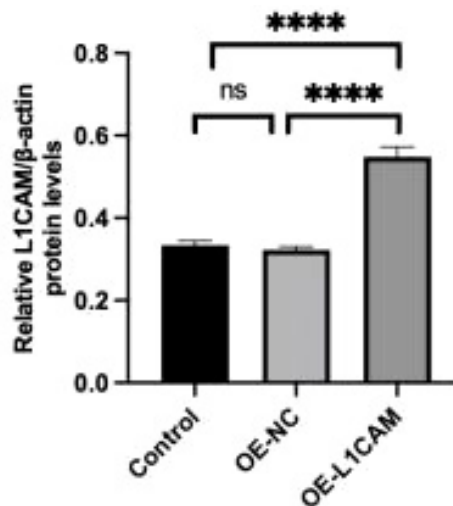


Figure 8. Expression results verified by WB when L1CAM gene was overexpressed.

3.3. Functional phenotype analysis

3.3.1. CCK-8 test results

The cell proliferation viability of each group was discovered by CCK-8 assay, and **Table 3** shows the results of CCK-8 assay. The differences in cell proliferation activity among all groups were shown in **Figure 9**. The results indicated that cell viability was significantly increased in the L1CAM overexpression transfection

group at 48 h, showing an approximately 17.0% increase compared to the control (set at 100%). The si-L1CAM transfection group showed a decreased cell viability, representing an approximately 13% reduction compared to the control (set at 100%).

Table 3. CCK-8 test results.

Grouping	OD value			Average value	Absolute value	Cell viability
Blank control	0.2044	0.2012	0.2004	0.2020	-	-
1: Blank control group	1.0418	1.0864	1.0168	1.0483	0.8463	100.00%
	1.0396	1.0755	1.0484	1.0545	0.8525	100.73%
	1.0386	1.0669	1.0328	1.0461	0.8441	99.74%
2: si-NC transfection group	1.0435	1.0327	1.0193	1.0318	0.8298	98.05%
	0.9422	0.8954	0.9349	0.9242	0.7222	85.33%
	1.0368	1.0500	1.0364	1.0411	0.8391	99.14%
3: si-L1CAM transfection group	1.1822	1.1639	1.1797	1.1753	0.9733	115.00%
	1.0417	1.0453	1.0915	1.0595	0.8575	101.32%
	0.9407	0.9631	0.9603	0.9547	0.7527	88.94%
4: Empty plasmid transfection group	1.0421	1.0376	1.0225	1.0341	0.8321	98.31%
	1.1748	1.2421	1.1942	1.2037	1.0017	118.36%
	1.0418	1.0536	1.0640	1.0531	0.8511	100.57%
5: L1CAM overexpression transfection group	0.9424	0.9270	0.9377	0.9357	0.7337	86.69%
	1.0701	1.0562	1.0806	1.0690	0.8670	102.44%
	1.1605	1.2388	1.1906	1.1966	0.9946	117.52%

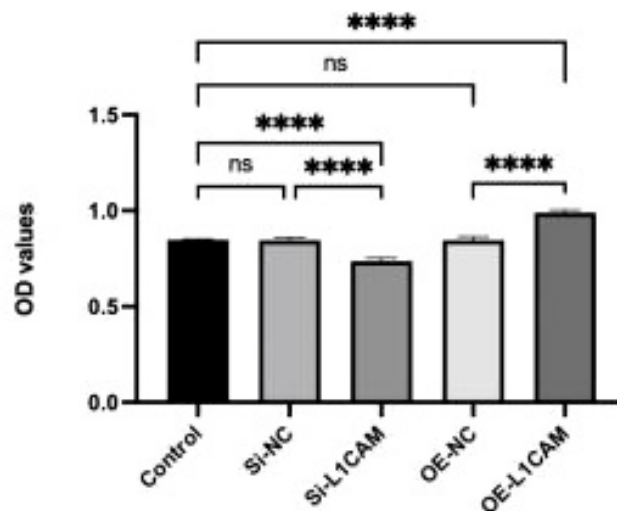


Figure 9. Differences in cell proliferation activity among all groups.

3.3.2. Results of migration statistics

Table 4 shows the statistical data of migration assay, Transwell assay showed that L1CAM significantly regulated the migration ability of cervical cancer cells. **Figure 10** shows the ratio of the number of migrating cells in each group compared with the

control group. Among them, both the si-NC transfection group and the empty plasmid transfection group were not notably different from the blank control group, indicating that the non-specific transfection operation had no effect on the migratory ability. The number of diatracted cells in the si-L1CAM transfection group was reduced by 20.3% contrasted with the control set, which suggests that L1CAM knockdown significantly impaired the migration ability. On the contrary, the number of diatracted cells in the overexpression of L1CAM group was elevated by 18.4% contrasted with the control set, suggesting that L1CAM was able to directly drive cell migration.

Table 4. Migration experiment statistics.

Migration cell count (24h)							
Blank control	Sample	Field of view 1	Field of view 2	Field of view 3	Field of view 4	Field of view 5	Average value
1: Blank control group	1-1	125	155	166	167	168	156
	1-2	122	142	157	161	175	151
	1-3	124	149	153	156	170	150
2: si-NC transfection group	2-1	127	149	153	168	176	155
	2-2	141	142	143	144	168	148
	2-3	128	147	155	172	175	155
3: si-L1CAM transfection group	3-1	115	120	123	126	137	124
	3-2	112	113	130	131	131	123
	3-3	104	111	113	128	129	117
4: Empty plasmid transfection group	4-1	129	143	148	160	164	149
	4-2	130	148	154	159	171	152
	4-3	150	154	156	157	158	155
5: L1CAM overexpression transfection group	5-1	164	180	181	181	205	182
	5-2	173	173	177	188	198	182
	5-3	163	164	164	194	198	177

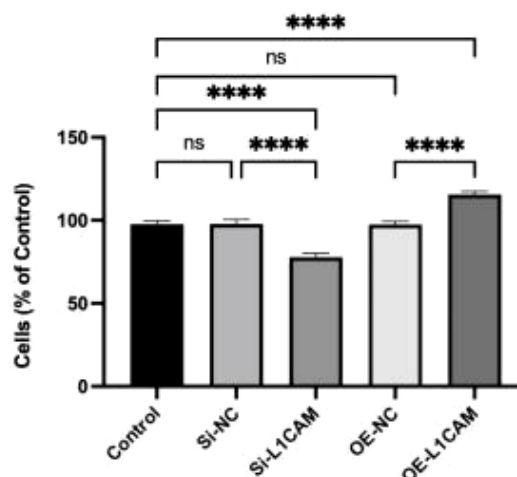


Figure 10. The proportion of the number of migrating cells compared to the control group.

3.3.3. Results of infestation statistics

Table 5 shows the statistics of invasion experiments. The proportion of the average number of invaded cells in each group compared with the blank control group is shown in **Figure 11**. Among them, both the si-NC transfection group and the empty plasmid transfection group were not significantly different from the blank control group, indicating that the non-specific transfection operation had no effect on the invasive ability. The average number of invaded cells in the si-L1CAM transfection group was significantly reduced to (40.0 ± 5.2) , which was 33.3% less than that of the blank control group, suggesting that L1CAM knockdown significantly inhibited the invasive ability. Meanwhile, the average number of invaded cells in the overexpression of L1CAM group was significantly elevated to (89.3 ± 6.4) , which increased by 48.8% compared with that of the blank control group, suggesting that the overexpression of L1CAM could significantly enhance the invasive ability.

3.3.4. Flow cells

Under the Annexin V-PE/7ADD double staining method, **Table 6** shows the data of flow cytometry experimental results. Differences of total apoptosis rate among all groups are shown in **Figure 12**. Analyzed data showed that L1CAM overexpression inhibited apoptosis, and the total apoptosis rate in the overexpression group was about 17.8% lower than that in the control group. Meanwhile, the total apoptosis rate in the si-L1CAM transfection group was an increase of approximately 102.4% over the control group. The average late apoptosis rate was significantly higher in the si-L1CAM transfection group, with an increase of 479% over the blank control group. The results suggest that L1CAM knockdown may accelerate the apoptotic process.

Table 5. Statistical data of invasion assay.

Invasion cell count (24h)							
Blank control	Sample	Field of view 1	Field of view 2	Field of view 3	Field of view 4	Field of view 5	Average value
1: Blank control group	A1	56	62	62	62	76	64
	A2	52	53	54	58	70	57
	A3	50	55	56	58	78	59
2: si-NC transfection group	B1	56	58	60	60	64	60
	B2	52	57	57	69	77	62
	B3	51	61	64	66	68	62
3: si-L1CAM transfection group	C1	33	38	41	46	55	43
	C2	27	35	39	39	53	39
	C3	23	27	35	23	54	38
4: Empty plasmid transfection group Grouping	D1	49	57	64	64	79	63
	D2	42	51	60	74	75	60
	D3	54	55	56	57	69	58

Continuation Table:

Invasion cell count (24h)							
Blank control	Sample	Field of view 1	Field of view 2	Field of view 3	Field of view 4	Field of view 5	Average value
5: L1CAM overexpression transfection group	E1	76	78	87	88	95	85
	E2	77	81	94	100	108	92
	E3	82	86	88	97	102	91

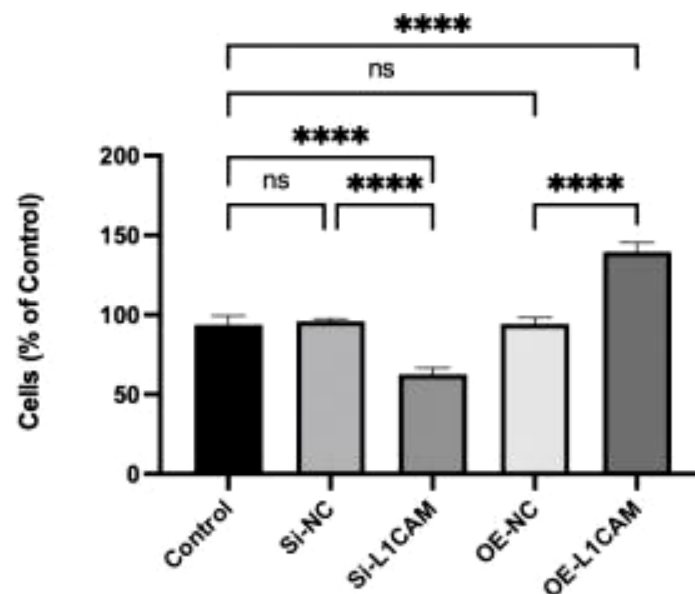


Figure 11. Proportion of average invasion cells compared to the blank control group.

Table 6. Flow cytometry experimental results data.

Grouping	Premature withering (Q1- LR)	Average premature death rate	Late withering (Q1-UR)	Average late apoptosis rate	Total apoptosis rate	The average total apoptosis rate
1: Blank control group	4.65%		1.60%		6.25%	
	4.52%	4.53%	1.84%	1.69%	6.36%	6.22%
	4.43%		1.63%		6.06%	
2: si-NC transfection group	3. 11%		3. 12%		6.23%	
	2.87%	2.94%	3.09%	3.10%	5.96%	6.05%
	2.85%		3. 10%		5.95%	
3: si-L1CAM transfection group	2.74%	2.81%	9.62%	9.78%	12.36%	12.59%
	2.85%		9.91%		12.76%	
	2.83%		9.81%		12.64%	
4: Empty plasmid transfection group	3.08%				5.96%	
	3. 14%	3.08%	2.95%	2.91%	6.09%	5.99%
	3.01%				5.92%	

Continuation Table:

Grouping	Premature withering (Q1- LR)	Average premature death rate	Late withering (Q1-UR)	Average late apoptosis rate	Total apoptosis rate	The average total apoptosis rate
5: L1CAM overexpression transfection group	3.29%		1.84%		5.13%	
	3.21%	3.27%	1.72%	1.84%	4.93%	5.11%
	3.31%		1.97%		5.28%	1

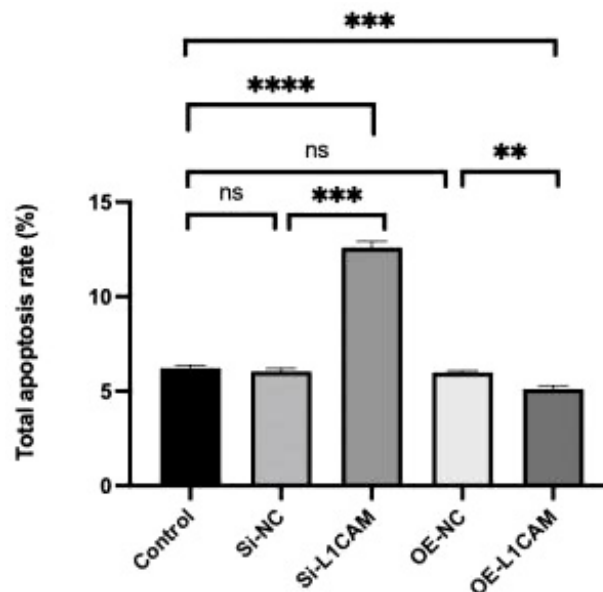


Figure 12. The results of total apoptosis rate by flow cytometry.

*** indicates $P < 0.001$, ** indicates $P < 0.01$.

4. Discussions

L1CAM is a type I transmembrane glycoprotein in the immunoglobulin superfamily with a molecular weight of 200–220 kD. It was identified in the nervous system and later found to be expressed significantly in colorectal, pancreatic, esophageal, Pulmonary neuroendocrine, prostate, ovarian and gastric cancer tissues [16].

Guo, M et al. conducted a meta-analysis of 17 studies covering 7146 patients to investigate the relationship between L1CAM expression and the clinical features of endometrial cancer. The results showed that L1CAM overexpression was significantly associated with decreased overall survival and disease-free survival, as well as adverse clinicopathologic features, such as higher disease grade, myxoid infiltration, lymphovascular interstitial infiltration, and lymph node metastasis, which further confirmed the promotional role of L1CAM in the progression of endometrial cancer [17]. Park et al. targeted mesonephric-like adenocarcinoma (MLA) in the uterus and immunostained 28 MLA patients for L1CAM and compared them with endometrioid (EC) and plasmacytoid (SC) carcinomas. The results showed that 53.6% of MLA cases had high L1CAM expression, which was more frequent than EC (7.5%) but less frequent than SC (78.9%). High L1CAM expression was associated with initial distant metastasis, lung metastasis, late initial stage and recurrence in MLA. Survival analysis showed that MLA patients with high L1CAM expression had worse disease-

free survival and overall survival, especially when the percentage of L1CAM staining was greater than or equal to 50%, and the patients had a significantly poorer prognosis, suggesting that L1CAM plays a facilitating role in the progression of MLA [18]. Consistent with the experimental results of this paper, the results of this study show that there is a high degree of synergy between the migration experiment data by comparing them with the invasion experiment data. Specifically, the si-L1CAM group showed a 20.3% decrease in migration and a 33.3% decrease in invasion, and the overexpression group showed an 18.4% increase in migration and a 48.8% increase in invasion, which demonstrates that L1CAM is able to promote metastasis through the dual mechanism of regulating cell motility and matrix degradation.

The mechanism of L1CAM's involvement in tumor cell motility can be explored from two aspects, the function of intercellular adhesion and driving cell motility, and the intervention on the expression of related genes [19].

Discussion of the mechanism of L1CAM on tumor cell proliferation invasion and metastasis can lead to the following points:

The L1CAM molecule is hydrolyzed by serine protease, trypsin, PC5A preprotein convertase, ADAM (10, 17), hydrolysis directly by ADAM10 or PMA-mediated hydrolysis by ADAM17, among others. Enzymatic hydrolysis generates the intracellular fragment L1-ICD and the extracellular fragment L1-200, which acts as an integrin ligand and can directly bind to extracellular integrins, (such as $\alpha\beta5$, $\alpha\beta1$, and $\alpha11\beta3$, etc.), thereby promoting both cell adhesion and motility [20]. EMT involved in tumor cells plays an important role in tumor invasion and metastasis, in addition, cleavage of adhesion molecules and induction of migratory phenotype are key signals for EMT initiation. It was found that L1CAM can trigger metastasis of epithelial cells by inhibiting the formation of calcium adhesion proteins and activating the β -catenin signaling pathway, which in turn initiates the EMT pathway [21]. At the same time, L1 extracellular fragments change the microenvironment in which cells are located, regulate the polarity and energetics of cells and tissues, and guide cell movement [19,22]. Ectodomain cleavage by ADAM10 is followed by intramembrane PS (presenilin)/ γ -secretase-dependent cleavage, leading to the generation of L1-ICD (L1 intracellular domain). L1-ICD may participate in transcriptional regulation of genes, such as those coding for cathepsinB, $\beta3$ integrin, CRABP II or HOXA9 (homeobox A9). L1-ICD can also participate in intranuclear signaling through ERK signalling pathway [22].

Furthermore, Zhang et al. [23] revealed that inhibiting L1CAM reversed Cisplatin Resistance of triple negative breast cancer cells by blocking AKT signaling pathway. Todorov et al. found that anti-L1CAM radioimmunotherapy with 161Tb could potentially overcome the radioresistance of L1CAM-expressing ovarian Cancer stem cells, providing a promising novel approach which could ultimately improve patient care [24]. These studies suggested that L1CAM inhibitors might well be a therapeutic avenue for tumours.

Therefore, this study deeply analyzes the role of L1CAM in cervical cancer proliferation, apoptosis and metastasis. And it tries to find an efficient L1CAM mechanism to be used in the future treatment of the disease.

Psychosocial and clinical relevance: Theoretical perspectives

The in vitro experimental design of the current study should be stressed by the fact that it requires no direct measure or validation of any psychological variables, patient-reported results, or neuroendocrine parameters. However, the results have implications at the theoretical and conceptual level of psycho-oncology that is

meaningful as we discuss them here in the spirit of integrative, biopsychosocial cancer research. One of the key determinants of the stage of the disease, the intensity of treatment, the probability of recurrence, and prognosis in general is tumor aggressiveness as measured in the present research by L1CAM-mediated augmentation of proliferation, invasion, migration, and resistance to apoptosis. In their turn, these clinical parameters are also considered to be among the strongest predictors of the psychological morbidity of patients with cervical cancer. Cyclically aggressive tumors with poor prognostic factors are associated with a significantly increased probability of clinically relevant anxiety, depression, fear of recurrence and reduced quality of life in comparison with those with less aggressive disease [25,26]. Specifically designed to measure these aspects of psychological distress and quality of life in cancer populations, validated instruments, including the Fear of Progression Questionnaire-Short Form (FoP-Q-SF), EORTC QLQ-C30, and the Functional Assessment of Cancer Therapy - General (FACT-G) should be used in future clinical correlation studies. Psychoneuroimmunologically, there could be a two-way process between psychological distress and the biology of tumours. Persistent psychological stress-initiated via HPA axis dysregulations and sympathoadrenal stimulations-increases blood glucocorticoids and catecholamines that may inhibit immune surveillance (including NK cell activity), stimulate pro-tumorigenic inflammatory responses (e.g., through IL-6, NF-Kb), and stimulate intracellular signaling cascades (e.g., ERK, AKT, MAPK pathways) that overlap with those that mediate L1CAM-mediated tumor invasion [27-29]. Although this pathway is not directly tested in our experiments, the convergence of stress-relevant neuroendocrine signaling and the L1CAM-controlled molecular processes are theoretically convergent, which makes it possible that the psychological stress causes the establishment of a permissive microenvironment in which the aggressive tumor behavior recorded in the current study is possible. These hypothetical psychobiological interactions may be explained by future studies, which combine in vitro models with stress hormone therapy, patient-reported outcome measures, biomarker profiling, etc. Moreover, in a clinical implementation sense, L1CAM expression profiling can indirectly have a usefulness in psychosocial risk stratification. Patients with tumors with high L1CAM expression -where there is higher invasive potential and a worse prognosis -could be a subgroup at increased risk of psychological distress and would benefit positively by psychological screening proactively and early-on incorporation of supportive psycho-oncological interventions, such as cognitive-behavioral therapy, peer support, and multidisciplinary palliative feedback. In the event that clinical samples are obtained, direct correlation analyses are to be conducted to test: (a) the relationship between the levels of L1CAM expression and scores on validated psychosocial measures (HADS, PHQ-9, GAD-7, Distress Thermometer);(b) how L1CAM defined disease aggressiveness affects quality of life using either EORTC QLQ-C30 or FACT-G; and (c) how prognosis awareness (based on biomarker status) is related to emotional burden, such as fear of recurrence and existential distress.

5. Conclusions

Recent studies have revealed that L1CAM is abnormally expressed in a variety of malignant tumors and is closely related to tumor invasion and metastasis. However, the mechanism of L1CAM in cervical cancer has not been fully elucidated. In this study, we systematically investigated the expression characteristics of L1CAM and its functional regulation in cervical cancer through molecular biology experiments.

The test study found that L1CAM expression levels of mRNA and protein were significantly higher in cervical cancer cell lines (HeLa, C-33A) than in normal cervical epithelial cells (HcerEpic). After knocking down L1CAM by siRNA, the proliferative, migratory and invasive capacities of cervical cancer cells were significantly reduced, the apoptosis rate was markedly increased. Conversely, overexpression of L1CAM significantly enhanced cellular proliferation, migration and invasion while suppressing apoptosis. This bi-directional regulatory effect suggests that L1CAM is not only a key driver of the malignant phenotype of cervical cancer, but also may regulate the level of tumor cell development through dynamic homeostasis. In addition to the molecular biology evidence, this study underscores the theoretical psychosocial implication of L1CAM-related tumor aggressiveness. Since the invasiveness and metastatic potential of the tumor are the leading causes of psychological distress, low quality of life, and negative psychosocial adaptation among the patients with cervical cancer, the L1CAM expression profile could be used as a validator to assess patients in the high-risk psychosocial category. Incorporation of such molecular biomarkers into clinical oncology could help to provide sooner psychosocial screening, prompt psycho-oncological intervention, and more comprehensive supportive care - eventually, this would help to provide holistic patient care that considers both the biological and psychological aspects of cervical cancer. There is a need to conduct future translational research to empirically investigate how the L1CAM expression levels, clinical outcomes, and patient-reported psychosocial well-being relate in prospective patient groups. In particular, prospective cohort studies are to be developed to recruit cervical cancer patients, obtain tumor tissue with the analysis of L1CAM expression (IHC or qPCR), and at the same time, administer validated psychosocial questionnaires (HADS, PHQ-9, GAD-7, FoP-Q-SF, EORTC QLQ-C30, Distress Thermometer) at The data would be applicable to test directly hypotheses about the relationship between L1CAM-mediated aggressiveness and psychological outcomes, and would constitute the evidence base of biomarker-informed psychosocial risk stratification and supportive care intervention.

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