Original Article
Middle East Journal of Cancer; April 2021; 12(2): 172-182

Prognostic and Clinicopathological Implications of NUSAP1, MELK and L1CAM Immunohistochemical Expression in Cervical Cancer

Mariem A. Elfeky*, MD, Ola A. Harb*♦, MD, Basant Sh El Shafaay, MD, Ahmed A. Obaya**, MD, Amr A. Awd***, MD, Mohamed M. Alkilany****, MD, Loay M. Gertallah****, MD**

*Department of Pathology, Faculty of Medicine, Zagazig University, Zagazig, Egypt
**Department of Clinical Oncology and Nuclear Medicine, Faculty of Medicine, Zagazig University, Zagazig, Egypt
***Department of Gynecology and Obstetrics, Faculty of Medicine, Zagazig University, Zagazig, Egypt
****Department of General Surgery, Faculty of Medicine, Zagazig University, Zagazig, Egypt

Abstract

Background: Evaluation of cancer cervix prognosis is highly needed for novel targeted therapy and improved outcomes. Nucleolar and spindle associated protein 1 (NUSAP1) is a novel biomarker that has roles in spindle formation and mitotic progression. Maternal embryonic leucine zipper kinase (MELK) is involved in cell cycle control and carcinogenesis. The L1 cell adhesion molecule (L1-CAM) plays an essential role in cell migration. This study aimed to investigate NUSAP1, MELK, and L1CAM immunohistochemical expression in cancer cervix tissues and detect their prognostic roles.

Method: In this prospective cohort study, we evaluated NUSAP1, MELK, and L1CAM expressions of sections from 62 cervical carcinoma cases using immunohistochemistry.

Results: NUSAP1, MELK, and L1CAM expression correlated with tumor high grade, advanced FIGO stage, poor survival rates, and higher recurrence rate after successful therapy (P<0.001).

Conclusion: Expression of NUSAP1, MELK, and L1CAM in cancer cervix was associated with poor prognosis.

Keywords: Cancer cervix, Immunohistochemistry, Prognosis, NUSAP1, MELK, L1CAM

Please cite this article as: Elfeky M, Harb O, El Shafaay B, Obaya A, Awd A, Alkilany M, et al. Prognostic and clinico-pathological implications of NUSAP1, MELK and L1CAM immunohistochemical expression in cervical cancer. Middle East J Cancer. 2021;12 (2):172-82. doi:10.30476/mejc. 2020.82884. 1115.

♦Corresponding Author:
Ola Harb, MD
Pathology Department Faculty of Medicine, Zagazig University, Zagazig As-sharkia Governorate, Egypt
Email: olaharb2015@gmail.com

This article has been partically presented in BGICC-Breast Gynecological and Immuno-Oncology International Cancer Conference 2020 and published in Tumori Journal: Vol 106, Issue 1 - suppl, April 2020.

Received: August 08, 2019; Accepted: August 26, 2020

Introduction

Cervical cancer is the second most prevalent female cancer worldwide.[1] The recent advancement in its surgical management, post-operative radiotherapy, and chemotherapy has resulted in improved outcomes and the five-year overall survival rate possibly reaching 80%. However, this rate has significantly decreased regarding the occurrence of lymph nodes, distant metastasis, or tumor recurrence.[2] Therefore, it is important to explore the molecular pathogenesis associated with the recurrence, invasion, spread, and progression of cervical carcinoma in order to identify novel therapeutic targets. Nucleolar and spindle associated protein 1 (NUSAP1) is able to bind microtubules and it plays important roles in spindle formation and mitotic progression.[3] Previous studies reported that NUSAP1 was up-regulated in several cancers.[4] Maternal embryonic leucine zipper kinase (MELK) is a conserved protein kinase related to cell cycle and located at chromosome 9p13.[5] It is involved in the control and regulation of the cell cycle, apoptosis, and carcinogenesis.[6] MELK was found to be expressed in many malignancies.[7] The L1 cell adhesion molecule (L1-CAM) was discovered as a protein with an essential role in cell migration and axon guidance in the nervous system.[8]

Previous reports suggested that the expression of NUSAP1, MELK, and L1CAM could be incriminated in the progression, recurrence, and poor outcome of many cancers. However, the expression of these three markers in cervical cancer has not been elucidated in detail.

The present article aimed at examining the tissue protein expression of NUSAP1, MELK, and L1CAM in cancer cervix patients, highlighting their prognostic and clinicopathological roles.

Materials and Methods

Our prospective cohort study included 62 patients with cervical carcinoma. They were admitted and operated in General Surgery Department and in Gynecology and Obstetrics Departments, Faculty of Medicine, Zagazig University. The operation was performed by total abdominal hysterectomy and bilateral salpingoophrectomy, with or without pelvic lymphadenectomy according to their stage, from May 2016 to May 2019. Samples were sent to Pathology Department, Faculty of Medicine, Zagazig University, where they were processed and diagnosed; grading and staging were done using the FIGO system.[9] None of the patients received pre-operative anticancer therapy.

The included patients were surgically managed according to their subtype followed by chemotherapy, radiotherapy, or combination therapies.

We followed our patients for progression, recurrence response to the currently used therapy, and three-year survival in Clinical Oncology and Nuclear Medicine Department and Medical Oncology Department.

The local institutional board of Faculty of Medicine, Zagazig University [ethics code: Zag00945, 2016] provided the ethical approval; the patients signed written informed consent for the use of tissue samples.

Immunohistochemistry

We performed immunohistochemistry as previously described by Hsu et al.;[10] the added primary antibodies were mouse polyclonal anti-NUSAP antibody (ab169083), mouse monoclonal anti-MELK antibody [2G2] (ab129373), and mouse monoclonal anti-L1CAM antibody [2C2] (ab24345) (1:200 dilution; Abcam, Cambridge, UK). Hematoxylin counterstain was done to detect the final stain.

Evaluation of the stain

Evaluation of NUSAP1, MELK, and L1CAM expression

We considered nuclear expression as positive NUSAP1 expression, cytoplasmic expression as positive MELK expression, while membranous and weak cytoplasmic expression as positive L1CAM expression.

We defined the extent of stained cells as follows: 0, if there were no positive tumor cells;1,

<10% positive tumor cells; 2, 10-35% positive tumor cells; 3, 35-75% positive tumor cells, and 4, > 75% positive tumor cells. We graded the stain intensity as follows: 0, if there was no stain;1 if there was weak stain; 2 if there was moderate stain; 3 if there was strong staining. We assessed the final staining immunoreactivity score through multiplying the intensity score by the extent of positive tumor cells score to evaluate the NUSAP1, MELK, and L1CAM protein expression levels. The final scores were 0-12. For statistical analysis, we defined specimens with a score of more than or equal to 6 as having high NUSAP1, MELK, and L1CAM expression; we defined samples with a score of less than 6 as having low NUSAP1, MELK and L1CAM expression.[2, 4]

Results

Patient clinicopathological characteristics

We included 62 cases of cancer cervix; 37 (59.7%) cases were >55 years old. 44 (71%) cases had squamous cell carcinoma and 18 (29%) cases had adenocarcinoma. 32 (51.6%) cases had lymph nodes metastases and 16 (25.8%) cases had distant metastases

Table 1 details the demographic and clinic-pathological findings of our patients.

The immunohistochemical results

NUSAP1 expression in tumor cells

We detected high levels of NUSAP1expression in 34 (54.8%) of cases. This expression correlated with older patients, high tumor grades, LN metastases, lympho-vascular invasion, advanced FIGO stage, distant metastases (P<0.001), and

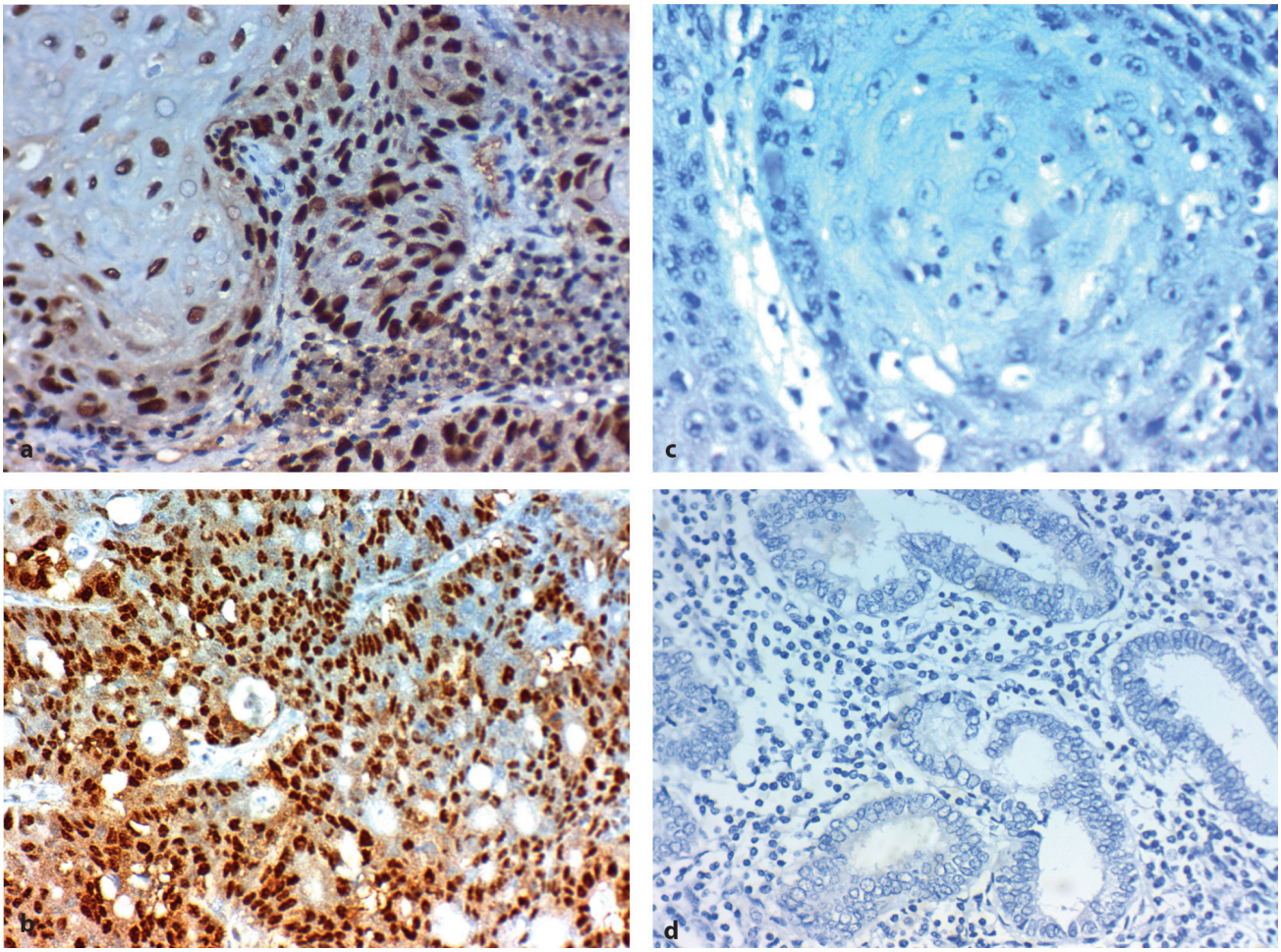

Figure 1. Immunohistochemical expression of NUSAP1 in cervical carcinoma: (a); high nuclear expression in poorly differentiated squamous cell carcinoma of the cervix ×400, (b); high nuclear expression in poorly differentiated adeno-carcinoma of the cervix ×400, (c); negative nuclear expression in well differentiated squamous cell carcinoma of the cervix ×400, (d); negative nuclear expression in well differentiated adeno-carcinoma of the cervix ×400.

Table 1. The correlation between NUSAP1, MELK, and L1CAM levels in the studied patients and their demographic and disease-specific characteristics

			NUSAP1			MELK			L1CAM		
			Low	High	P¥	Low	High	P¥	Low	High	P¥
	N = 62	%	N=28 (45.2%)	N=34 (54.8%)		N=28 (45.2%)	N=34 (54.8%)		N=38 (61.3%)	N=24 (38.7%)	
Age group											
≤ 55 years old	25	40.3	26 (92.7)	11 (32.4)	<0.001*	16 (57.1)	9 (26.5)	0.014*	18 (47.4)	7 (29.2)	0.155
>55 years old	37	59.7	2 (7.1)	23 (67.6)		12 (42.9)	25 (73.5)		20 (52.6)	17 (70.8)	
Histopathology											
Squamous cell carcinoma	44	71	19 (67.9)	25 (73.5)	0.78	19 (67.9)	25 (73.5)	0.78	26 (68.4)	18 (75)	0.775
Adenocarcinoma	18	29	9 (32.1)	9 (26.5)		9 (32.1)	9 (26.5)		12 (31.6)	6 (25)	
Size											
<4cm	6	9.7	6 (21.4)	0 (0)	0.006*	6 (21.4)	0 (0)	0.006*	6 (15.8)	0 (0)	0.073
≥4cm	56	90.3	22(78.6)	34 (100)		22 (78.6)	34 (100)		32 (84.2)	24 (100)	
Grade											
I	10	16.1	10 (35.7)	0 (0)	<0.001*	10 (35.7)	0 (0)	<0.001*	10 (26.3)	0 (0)	<0.001*
II	38	61.3	17 (60.7)	21 (61.8)		16 (57.1)	22 (64.7)		26 (68.4)	12 (50)	
III	14	22.6	1 (3.6)	13 (38.2)		2 (7.1)	12 (35.3)		2 (5.3)	12 (50)	
LVSI											
Absent	44	71	27 (96.4)	17 (50)	<0.001*	26 (92.9)	18 (52.9)	0.001*	35 (92.1)	9 (37.5)	<0.001*
Present	18	29	1 (3.6)	17 (50)		2 (7.1)	16 (47.1)		3 (7.9)	15 (62.5)	
Lymph node											
Absent	30	48.4	22 (78.6)	8 (23.5)	<0.001*	22 (78.6)	8 (23.5)	0.002*	26 (68.4)	4 (16.7)	<0.001*
Present	32	51.6	6 (21.4)	26 (76.5)		6 (21.4)	26 (76.5)		6 (31.6)	20 (83.3)	
Distant metastasis											
Absent	46	74.2	27 (96.4)	19 (55.9)	<0.001*	20 (71.4)	10 (29.4)	0.003*	35 (92.1)	11 (45.8)	<0.001*
Present	16	25.8	1 (3.6)	15 (44.1)		8 (28.6)	24 (70.6)		3 (7.9)	13 (54.2)	
Stage											
I	6	9.7	6 (21.4)	0 (0)	<0.001*	6 (21.4)	0 (0)	0.001*	6 (15.8)	0 (0)	0.001*
II	24	38.7	16 (57.1)	8 (23.5)		14 (50)	10 (29.4)		20 (52.6)	4 (16.7)	
III	16	25.8	5 (17.9)	11 (32.4)		6 (21.4)	10 (29.4)		9 (23.7)	7 (29.2)	
IV	16	25.8	1 (3.6)	15 (44.1)		2 (7.1)	14 (41.2)		3 (7.9)	13 (54.2)	
NULP											
Low	28	45.2				26 (92.9)	2 (5.9)	<0.001*	28 (73.7)	0 (0)	<0.001*
High	34	54.8				2 (7.1)	32 (94.1)		10 (26.3)	24 (100)	
MELK											
Low	28	45.2	26 (92.9)	2 (5.9)	<0.001*				28 (73.7)	0 (0)	<0.001*
High	34	54.8	2 (7.1)	32 (94.1)					10 (26.3)	24 (100)	
L1CAM											
Low	38	61.3	28 (100)	10 (29.4)	<0.001*	28 (100)	10 (29.4).	<0.001*			
High	24	38.7	0 (0)	24 (70.6)		0 (0)	24 (70.6)				

*P<0.05 is statistically significant ¥ Chi square test; NUSAP1: nucleolar and spindle associated protein 1; MELK: maternal embryonic leucine zipper kinase; L1CAM: L1 cell adhesion molecule

large cancer size (P=0.006). There was no statistically significant relationship between NUSAP1 expression and histopathological subtype (Table 1, Figure 1).

Progression, recurrence, therapy response, and survival rates in correlation to NUSAP1 expression

There was a statistically significant difference among the patients concerning NUSAP1 levels, treatment response, recurrence, disease-free survival, and overall survival. Patients with high levels of NUSAP1 expression had poor survival rates, poor therapy response, and higher recurrence rates after therapy (P<0.001) (Tables 2-4, Figure 4).

MELK expression

We found high levels of MELK expression in 34 (54.8%) of cases, which was positively related to older patients, high tumor grades, lympho-vascular invasion, advanced FIGO stage (P=<0.001), large cancer size (P=0.006), presence of LN metastases (P=0.002) and distant metastases (P=0.003). There was no statistically significant association between MELK expression and histopathological subtype (Table 1, Figure 2).

Progression, recurrence, therapy response, and survival rates in correlation to MELK expression

There was a statistically significant difference

among the patients concerning the level of MELK, treatment response, recurrence, disease-free survival, and overall survival. Patients with high levels of MELK expression had poor survival rates, poor therapy response, and higher recurrence rates following therapy (P<0.001) (Tables 2-4, Figure 4).

L1CAM expression

We observed high levels of L1CAM expression in 24 (38.7%) of the cases; these levels were positively related to high tumor grades, lympho-vascular invasion, advanced FIGO stage, LN metastases, and distant metastases (P=≤0.001). There was no statistically significant relationship among L1CAM expression, patients' age, size of the tumor, or histopathological subtype (Table 1, Figure 3).

Progression, recurrence, therapy response, and survival rates in correlation to L1CAM expression

There was a statistically significant difference in L1CAM levels, treatment response, recurrence, disease-free survival, and overall survival. Patients with high levels of L1CAM expression had poor survival rates, poor therapy response, and higher recurrence rates after therapy (P<0.001) (Tables 2 and 3, Figure 4).

There was a statistically significant difference between mortality and each of the three markers. High NURP resulted in 27.18-fold increase in mortality, high MELK led to 64.8-fold increase, and high L1CAM entailed a 19.568-fold rise in mortality.

There was a statistically significant, positive

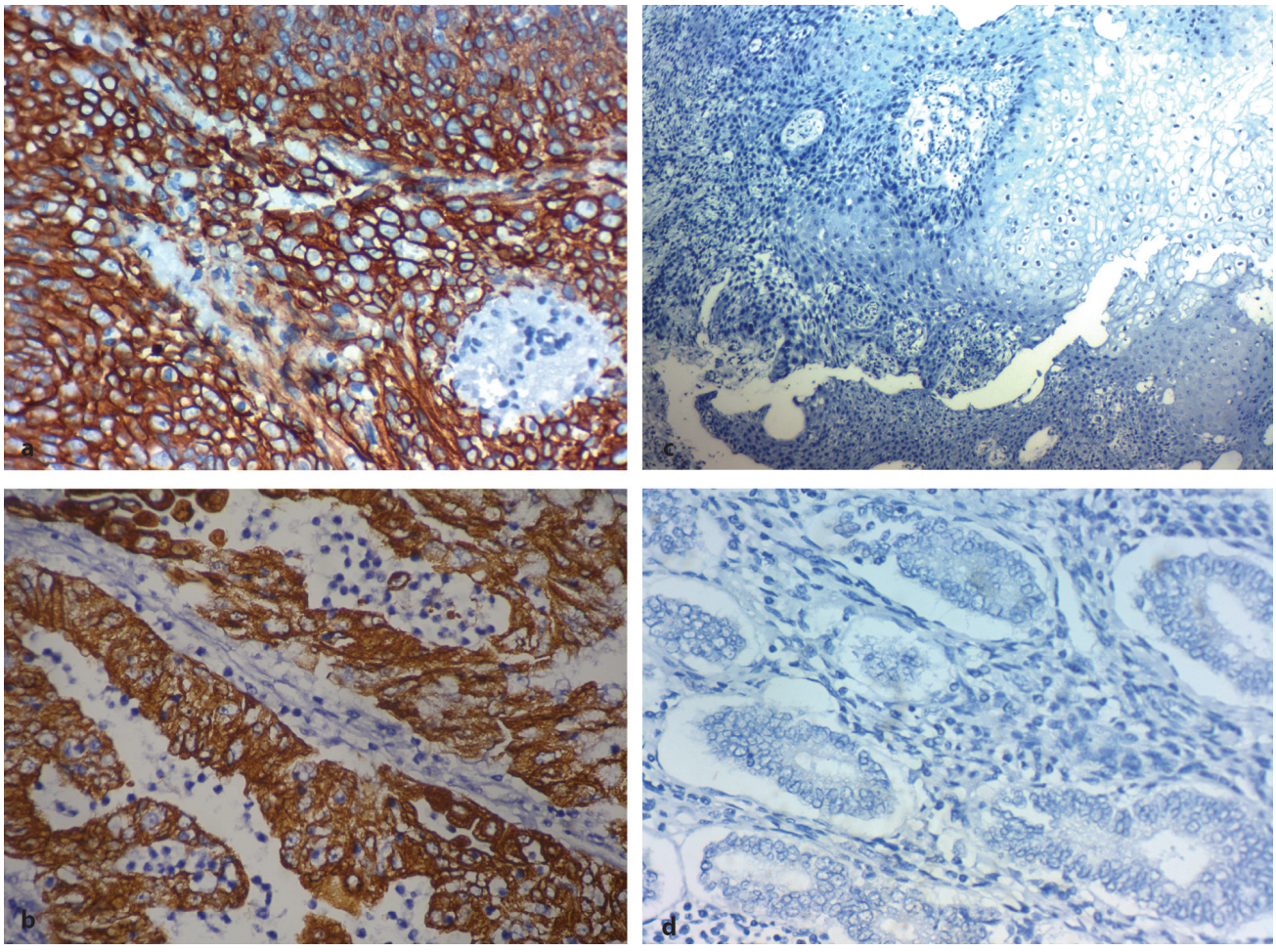

Figure 2. Immunohistochemical expression of MELK in cervical carcinoma: (a) high cytoplasmic expression in poorly-differentiated squamous cell carcinoma of the cervix (×400), (b) high cytoplasmic expression in poorly-differentiated adenocarcinoma of the cervix (×400), (c) negative cytoplasmic expression in well-differentiated squamous cell carcinoma of the cervix (×400), (d) negative cytoplasmic expression in well-differentiated adenocarcinoma of the cervix (×400).

Table 2. Distribution of the studied patients according to treatment-specific characteristics and their outcomes

	N = 62	%
Treatment		
Surgery	13	21
Surgery and radiotherapy	10	16.1
Surgery and chemotherapy	17	27.4
Surgery, radiotherapy and chemotherapy	14	22.6
Radiotherapy	4	6.5
Chemotherapy	4	6.5
Response		
PD	37	59.7
SD	4	6.5
PR	7	11.3
CR	14	22.6
OAR	41	66.1
NR	21	33.9
Outcome		
Alive	37	59.7
Dead	25	40.3
Disease free survival (months) (N=16)		
Mean ± SD	29.03 ± 6.49	
Range	16 - 36	
Overall survival (months) (N=10)		
Mean ± SD	27.68 ± 9.18	
Median (Range)	30 (10 – 36)	

PD: progressive disease; SD: stable disease; PR: partial response; CR: complete response; NR: no response; OAR: overall response

association among NUSAP1, MELK, and L1CAM Phi correlation coefficient= + 0.87 and + 0.721, respectively (P<0.001).

Discussion

The present study reported the increased expression of NUSAP1 in cervical cancer, which was related to poor clinical outcomes and unfavorable pathological parameters, mainly through promoting invasion and metastasis. Additionally, we found that NUSAP1 overexpression had a correlation with increased lymph node and distant metastasis. Our results are in line with Li et al. regarding NUSAP1 expression in cancer cervix.[2]

Similarly, Gordon et al. showed that NUSAP1 expression correlated with unfavorable prognosis of different cancers.[11]

NUSAP1 was previously shown to be able to activate the Wnt/ β-catenin signaling pathway. Therefore, it contributes to the metastatic properties and activation of cancer stem cell-like features in cervical cancer cells and several other cancers; this also possibly explains our results.[2, 12,13] Additionally, the Wnt/β-catenin pathway plays a significant role in cancer progression via activating epithelial mesenchymal transition (EMT), contributing to the invasion and metastasis of many types of cancer.[14, 15]

Okamoto et al. provided another explanation for the role of NUSAP1 in cancer.[2] They demonstrated that the inhibition of NUSAP1 could inhibit the proliferation of cancer cells and enhance the paclitaxel related anticancer properties through the activation of apoptotic pathways. Moreover, the knockdown of NUSAP1 led to cell cycle arrest at G2/M phase, suppressing glioma cell proliferation.[17] Additionally, NUSAP1 depletion I colon cancer cells inhibited cell proliferation, migration, invasion, and EMT through the induction of apoptosis and inhibition of DNA methyltransferase 1 expression.[2] Roy et al. reported similar roles for NUSAP1 in the cells of hepatocellular carcinoma.[18] The foregoing studies showed that increased NUSAP1 expression led to an increase in the malignant criteria of

many cancers, hence the unfavorable patient outcomes. Here, we clarified the prognostic roles of NUSAP1 expression in the tissues of the carcinoma of the cervix, which is in accordance with the previously detailed findings.

There has been a marked improvement in cervical cancer treatment; however, invasion and metastases are still major obstacles. In this regard, we showed that Wnt/β-catenin signaling activation, caused by NUSAP1, entailed cervical cancer progression by activating the EMT. Thus, targeting such pathways might represent a hopeful novel anticancer therapy. Li et al. used XAV-939 (an inhibitor of WNT signaling pathway) to inhibit the transcription of catenin.[2] They also added XAV-939 to inhibit cancer stem cells, subsequently leading to the inhibition of metastasis in cancer cervix cells.

To prove the multiple roles of NUSAP1 in cervical cancer progression, we assessed the expression of another marker (MELK) and correlated its expression with clinicopathological and prognostic parameters and NUSAP1 expression. Overexpression of MELK was related to the poor prognosis of many malignancies, cancer invasion, metastasis, and recurrence.[2] The present study showed that MELK was over-expressed in cervical cancer tissues; moreover, its expression levels had a correlation with unfavorable pathological and clinical parameters, which is consistent with Wang et al.[4] MELK may play an important role in the oncogenesis and progression of cervical cancer; it could also be a novel predictive and prognostic marker for patient

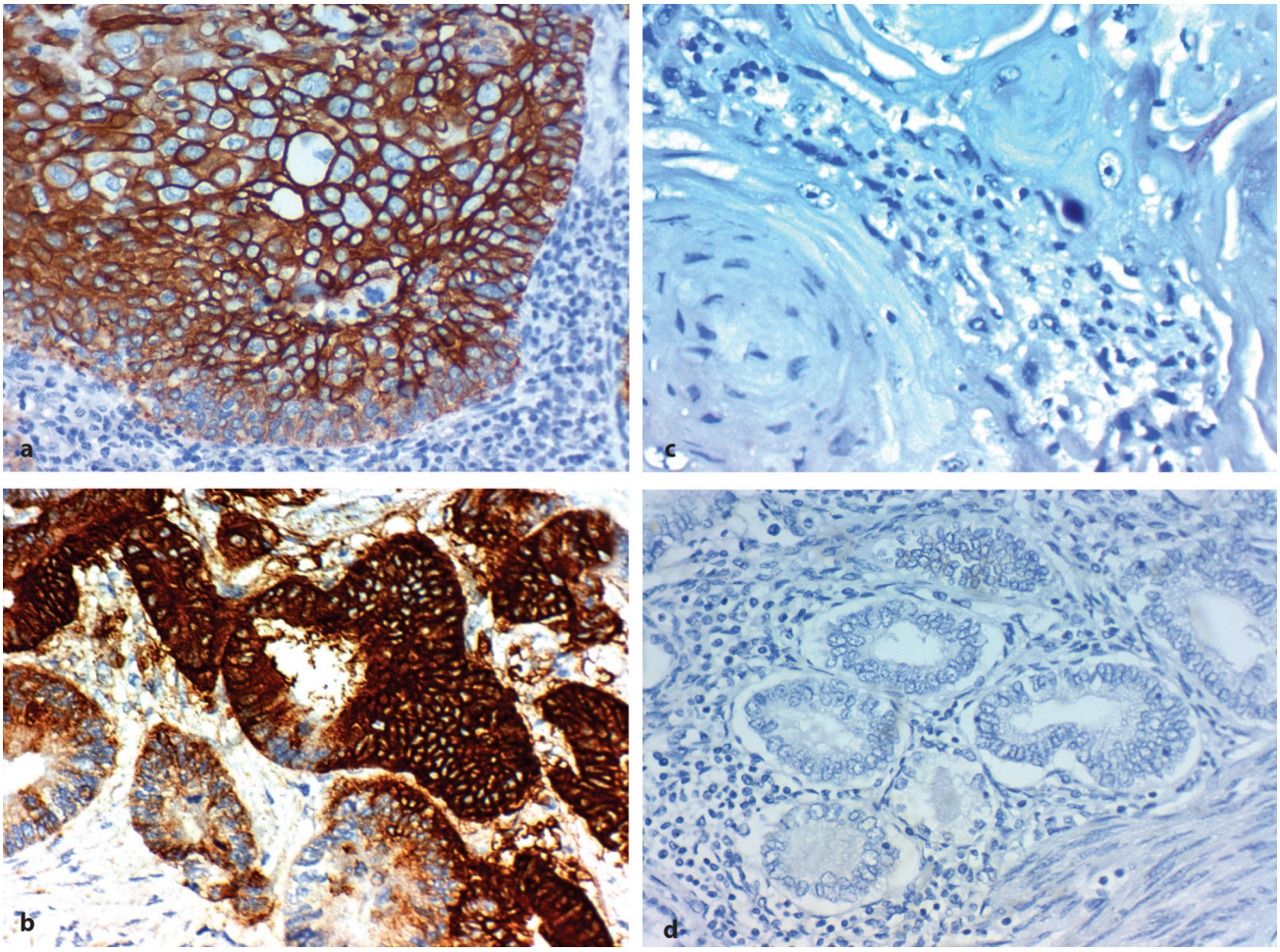

Figure 3. Immunohistochemical expression of L1CAM in cervical carcinoma: (a) high membranous expression in poorly-differentiated squamous cell carcinoma of the cervix (×400), (b) high membranous expression in moderately-differentiated adenocarcinoma of the cervix (×400), (c) negative membranous expression in well-differentiated squamous cell carcinoma of the cervix (×400), (d) negative membranous expression in well-differentiated adenocarcinoma of the cervix (×400).

Table 3. The correlation between NUSAP1, MELK, and L1CAM levels in the studied patients and treatment-specific characteristics and their outcomes

	NUSAP1			MELK			L1CAM		
	Low N=28 (45.2%)	High N=34 (54.8%)	*P*	Low N=28 (45.2%)	High N=34 (54.8%)	*P*	Low N=38 (61.3%)	High N=24 (38.7%)	*P*
Treatment response									
CR	27 (96.4)	10 (29.4)	<0.001*	26 (92.9)	11 (32.4)	<0.001*	31 (81.6)	6 (25)	<0.001*
PR	1 (3.6)	3 (8.8)		2 (7.1)	2 (5.9)		4 (10.5)	0 (0)	
SD	0 (0)	7 (20.6)		0 (0)	7 (20.6)		2 (5.3)	5 (20.8)	
PD	0 (0)	14 (41.2)		0 (0)	14 (41.2)		1 (2.6)	13 (54.2)	
Response									
OAR	28 (100)	13 (38.2)	<0.001*	28 (100)	13 (38.2)	<0.001*	35 (92.1)	6 (25)	<0.001*
NR	0 (0)	25 (61.8)		0 (0)	21 (61.8)		3 (7.9)	18 (75)	
Recurrence (n=38)									
Absent	16 (59.3)	1 (9.1)	<0.001*	17 (63)	0 (0)	<0.001*	17 (53.1)	0 (0)	<0.001*
Present	11 (40.7)	10 (90.9)		10 (37)	11 (100)		15 (46.9)	6 (100)	
Disease free survival									
Mean ± SD	31.41±5.62	23.18 ± 4.58	<0.001*∞	33.69 ± 3.4	29.36±5.7	<0.001*∞	32.88±4.6	31.33±6.81	0.003*∞
Range	17 - 36	16 - 30		22 - 36	16 - 36		20 - 36	30 - 34	
Overall survival									
Median	36	28	<0.001*#	32	23	<0.001*#	30	25	<0.001*#
Range	22 - 36	10 - 36		16 - 36	17 - 30		16 - 36	23 - 28	

t Independent sample t test, ∞Z Mann Whitney test, *P<0.05 is statistically significant, ¥ chi square test, #independent sample t test; PD: progressive disease; SD: stable disease; PR: partial response; CR: complete response; NR: no response; OAR: overall response; NUSAP1: nucleolar and spindle associated protein 1; MELK: maternal embryonic leucine zipper kinase ; L1CAM: L1 cell adhesion molecule

outcomes and a therapeutic target for many cancers.[2]

MELK was shown to stimulate the proliferation of tumor cells, increase mitosis, and play an essential role in the P53-P21 apoptotic pathway.[20, 21]

Therapeutic agents targeting MELK were previously evaluated; for instance, OTSSP167 (a selective MELK inhibitor) was reported to inhibit the proliferation of cancer cells, but its role against cervical cells is not clarified.[22]

Additionally, Kohler et al. found that using OTSSP167 as an MELK inhibitor, led to the induction of G2/M cell cycle arrest, which inhibited cell proliferation and induced apoptosis.[23] Wang et al. used different concentrations of OTSSP167 to inhibit MELK in cancer cervix cells and determine its role in the induction of proliferation; P53 and cleaved caspase-3 (apoptosis-related proteins) showed that inhibited MELK affected the proliferation and invasion of many cervical cancer cells and promoted apoptosis and cell senescence.[4]

Targeting MELK by its inhibitor MELK-T1 reduced the tolerance to DNA damage and made the cancer cells more sensitive to DNA damaging agents or radiotherapy.[24] Therefore, inhibition of MELK could be considered as a novel and favorable strategy for improving the response to chemoradiotherapy in cancers.[4] We found a positive association between NUSAP1 and MELK expression in cervical carcinoma tissue. This is because both markers correlated with controlling tumor cell apoptosis, cell cycle progression, and cancer metastases. To confirm the correlation, we assessed the expression of another adhesion molecule (L1CAM) and specified the relationship between its expression, clinicopathological, and prognostic finding and patient outcomes.

We investigated the association between L1CAM expression in cervical cancer cells and patients' survival; we also examined the relationship between L1CAM expression and cancer progression. Our findings showed that L1CAM expression was related to unfavorable pathological parameters and poor outcomes; it was also a strong predictor of worse recurrence-free survival and overall survival rates in cervical cancer patients. Our results are similar to previous studies on cervical cancer and other malignancies.[25-31] As explained by Altevogt et al., L1CAM could be considered as a promising novel therapeutic target against cancers.[30] The role of L1CAM in cancer progression might be attributed to its role in EMT.[25] Zecchini et al. described the dual role of L1CAM in cancer cells.[26] It supported the adhesion between cells and activated apoptosis in non-neoplastic ovarian cells, while inhibiting adhesion and apoptosis and inducing cell proliferation, invasion, and metastases in ovarian cancer cells. Over-

expression of L1CAM in cervical cancer increased the rates of proliferation, invasion, and migration. EMT induction by L1CAM in cervical cancer is of extreme importance as it leads to resistance to the currently used therapies.[32] Further studies are needed to assess the role of L1CAM in inducing EMT in cervical cancer.[26]

We performed this study to assess the expression of EMT with other involved markers. We found a positive correlation between L1CAM and NUSAP1 which is associated with EMT and stem cell properties in cervical cancer tissues; we also observed a positive association between L1CAM and MELK expression, which is associated with disturbances in cell cycle control, apoptosis, and progression of cervical cancer. This explains the overlapping roles of the three markers and the recurrence, spread, progression, and dismal outcomes of cervical cancer patients. Therapeutic targets against those markers could be promising in the management of cervical cancer, improving its prognosis, and reducing its invasion, metastases, and recurrence.

Conclusion

Metastasis is the main obstacle against the success of therapy in cervical cancer patients; therefore, novel mechanisms and biomarkers for predicting and controlling metastasis might reduce the progression of cervical carcinoma and improve the patients' prognosis. The results of our study clarified the roles of NUSAP1, MELK, and L1CAM in EMT, cell cycle progression, invasion, and metastases; our findings further pointed to their possible use as predictive and prognostic markers for cervical cancer patients and identified patients with NUSAP1, MELK, and L1CAM overexpression, who might benefit from targeted therapies against such markers in addition to the traditional treatment.

Limitations of the study

Small number of cases, short follow-up period, and a single method for evaluating the biomarkers

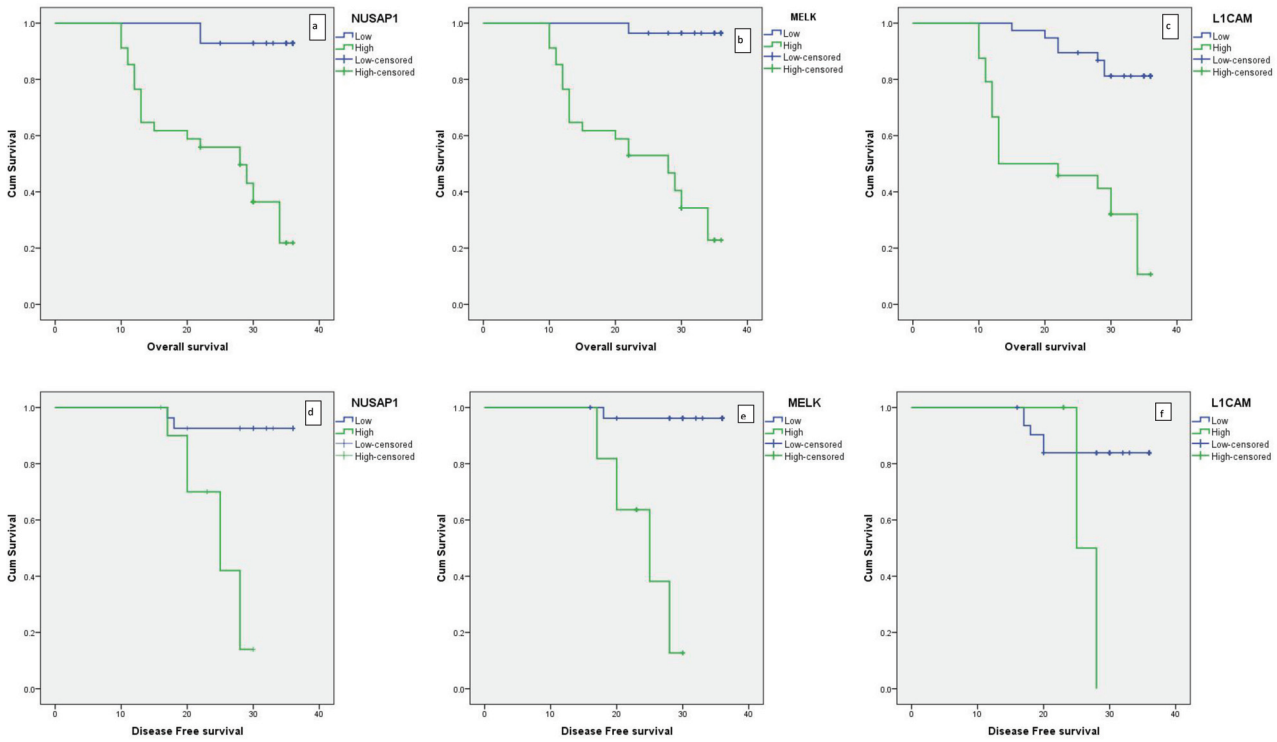

Figure 4. Kaplan Meir survival curves of OS rate and DFS of the studied cervical carcinoma patients: (a-c) OS of the studied cervical carcinoma cases stratified according to NUSAP1, MELK, and L1CAM expression respectively, (d-f) DFS rates of the studied cervical carcinoma cases stratified according to NUSAP1, MELK, and L1CAM expression, respectively.

NUSAP1: nucleolar and spindle associated protein 1; MELK: maternal embryonic leucine zipper kinase; L1CAM: L1 cell adhesion molecule; OS: overall survival; DFS: disease-free survival

Table 4. The correlation between NUSAP1, MELK, L1CAM expression levels in the studied patients and their outcomes

Patients' outcomes	NUSAP1		MELK		L1CAM	
	High N=34 (%)	Low N=28 (%)	High N=34 (%)	Low N=28 (%)	High N=24 (%)	Low N=38 (%)
Outcome						
Dead	23 (67.6)	2 (7.1)	24 (70.6)	1 (3.6)	18 (75)	7 (18.4)
Alive	11 (32.4)	26 (92.9)	10 (29.4)	27 (96.4)	6 (25)	31 (81.6)
X^2	Fisher		Fisher		19.568	
P	<0.001**		<0.001**		<0.001**	
Odds ratio	27.18		64.8		13.29	
95% confidence interval	5.45-136.68		7.72-544.14		3.86-45.7	

OR: odds ratio; CI: confidence interval; NUSAP1: Nucleolar and spindle associated protein 1; MELK: Maternal embryonic leucine zipper kinase ; L1CAM: L1 cell adhesion molecule, ** $P \leq 0.001$ is statistically highly significant

were among the limitations of the present study.

Due to the many overlapping action mechanisms of NUSAP1, MELK, and L1CAM, future studies are to accurately detect the mechanisms of metastasis induced by their up-regulation through assessing their molecular expression.

Conflicts of Interest

None declared.

References

1. Li F, Wang Z, Lu G. TRIM28 promotes cervical cancer growth through the mTOR signaling pathway. *Oncol Rep.* 2018;39(4):1860-6.
2. Li H, Zhang W, Yan M, Qiu J, Chen J, Sun X, et al. Nucleolar and spindle associated protein 1 promotes metastasis of cervical carcinoma cells by activating Wnt/β-catenin signaling. *J Exp Clin Cancer Res*. 2019;38(1):33.
3. Ribbeck K, Raemaekers T, Carmeliet G, Mattaj IW. A role for NuSAP in linking microtubules to mitotic chromosomes. *Curr Biol.* 2007;17(3):230-6.
4. Wang J, Wang Y, Shen F, Xu Y, Zhang Y, Zou X, et al. Maternal embryonic leucine zipper kinase: A novel biomarker and a potential therapeutic target of cervical cancer. *Cancer Med.* 2018;7(11):5665-78. doi:10.1002/cam4.1816.
5. Pitner MK, Taliaferro JM, Dalby KN, Bartholomeusz C. MELK: a potential novel therapeutic target for TNBC and other aggressive malignancies. *Expert Opin Ther Targets*. 2017;21(9):849-59. doi:10.1080/14728222.2017.1363183.
6. Guan S, Lu J, Zhao Y, Yu Y, Li H, Chen Z, et al. MELK is a novel therapeutic target in high-risk neuroblastoma. *Oncotarget.* 2017;9(2):2591-602. doi:10.18632/oncotarget.23515.
7. Wu J, Hong C, Huang W, Wei X, Zhang F, Zhuang Y, et al. L1 Cell adhesion molecule and its soluble form sL1 exhibit poor prognosis in primary breast cancer patients. *Clin Breast Cancer.* 2018;18(5):e851-e861. doi:10.1016/j.clbc.2017.12.011.
8. Pecorelli S, Zigliani L, Odicino F. Revised FIGO staging for carcinoma of the cervix. *Int J Gynaecol Obstet.* 2009;105(2):107-8.
9. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem*. 1981;29(4):577-80.
10. Gordon CA, Gong X, Ganesh D, Brooks JD. NUSAP1 promotes invasion and metastasis of prostate cancer. *Oncotarget.* 2017;8(18):29935-50.
11. Lan K, Zhao Y, Fan Y, Ma B, Yang S, Liu Q, et al. Sulfiredoxin may promote cervical cancer metastasis via Wnt/β-Catenin signaling pathway. *Int J Mol Sci.* 2017;18(5):917.
12. Sha J, Han Q, Chi C, Zhu Y, Pan J, Dong B, et al. PRKAR2B promotes prostate cancer metastasis by activating Wnt/β-catenin and inducing epithelial-mesenchymal transition. *J Cell Biochem.* 2018;119(9): 7319-27.
13. Yeh HW, Hsu EC, Lee SS, Lang YD, Lin YC, Chang CY, et al. PSPC1 mediates TGF-β1 autocrine signalling and Smad2/3 target switching to promote EMT, stemness and metastasis. *Nat Cell Biol.* 2018;20(4):479-91.
14. Wang T, Wu H, Liu S, Lei Z, Qin Z, Wen L, et al. SMYD3 controls a Wnt-responsive epigenetic switch for ASCL2 activation and cancer stem cell maintenance. *Cancer Lett.* 2018;430:11-24.
15. Okamoto A, Higo M, Shiiba M, Nakashima D, Koyama T, Miyamoto I, et al. Down-regulation of nucleolar and spindle-associated protein 1 (NUSAP1) expression suppresses tumor and cell proliferation and enhances anti-tumor effect of paclitaxel in oral squamous cell

carcinoma. *PLoS One.* 2015;10(11): e0142252.
16. Zhu T, Xie P, Gao YF, Huang MS, Li X, Zhang W, et al. Nucleolar and spindle-associated protein 1 is a tumor grade correlated prognosis marker for glioma patients. *CNS Neurosci Ther.* 2018;24(3):178-86.
17. Han G, Wei Z, Cui H, Zhang W, Wei X, Lu Z, et al. NUSAP1 gene silencing inhibits cell proliferation, migration and invasion through inhibiting DNMT1 gene expression in human colorectal cancer. *Exp Cell Res.* 2018;367(2):216-21.
18. Roy S, Hooiveld GJ, Seehawer M, Caruso S, Heinzmann F, Schneider AT, et al. microRNA 193a-5p regulates levels of nucleolar- and spindle-associated protein 1 to suppress hepatocarcinogenesis. *Gastroenterology.* 2018;155(6):1951-66. e26.
19. Li S, Li Z, Guo T, Xing XF, Cheng X, Du H, et al. Maternal embryonic leucine zipper kinase serves as a poor prognosis marker and therapeutic target in gastric cancer. *Oncotarget.* 2016;7(5):6266-80.
20. Giuliano CJ, Lin A, Smith JC, Palladino AC, Sheltzer JM. MELK expression correlates with tumor mitotic activity but is not required for cancer growth. *Elife.* 2018;7:e32838.
21. Matsuda T, Kato T, Kiyotani K, Tarhan YE, Saloura V, Chung S, et al. p53-independent p21 induction by MELK inhibition. *Oncotarget.* 2017;8(35):57938-47.
22. Chung S, Nakamura Y. MELK inhibitor, novel molecular targeted therapeutics for human cancer stem cells. *Cell Cycle.* 2013;12(11):1655-6.
23. Kohler RS, Kettelhack H, Knipprath-Meszaros AM, Fedier A, Schoetzau A, Jacob F, et al. MELK expression in ovarian cancer correlates with poor outcome and its inhibition by OTSSP167 abrogates proliferation and viability of ovarian cancer cells. *Gynecol Oncol.* 2017;145(1):159-66.
24. Beke L, Kig C, Linders JT, Boens S, Boeckx A, Heerde E, et al. MELK-T1, a small-molecule inhibitor of protein kinase MELK, decreases DNA-damage tolerance in proliferating cancer cells. *Biosci Rep.* 2015;35(6):e00267.
25. Schrevel M, Corver W, Vegter M, Ter Haar N, Dreef E, Beltman J, et al. L1 cell adhesion molecule (L1CAM) is a strong predictor for locoregional recurrences in cervical cancer. *Oncotarget.* 2017;8(50):87568-81.
26. Zecchini S, Bianchi M, Colombo N, Fasani R, Goisis G, Casadio C, et al. The differential role of L1 in ovarian carcinoma and normal ovarian surface epithelium. *Cancer Res.* 2008;68(4):1110-8.
27. Zeimet AG, Reimer D, Huszar M, Winterhoff B, Puistola U, Azim SA, et al. L1CAM in early-stage type I endometrial cancer: results of a large multicenter evaluation. *J Natl Cancer Inst.* 2013;105(15):1142-50.
28. Trietsch MD, Oonk MH, Hawinkels LJ, Bor R, van Eendenburg JD, Ivanova Z, et al. Prognostic value and clinicopathologic characteristics of L1 cell adhesion molecule (L1CAM) in a large series of vulvar squamous cell carcinomas. *Oncotarget.* 2016;7(18):26192-205.
29. Bondong S, Kiefel H, Hielscher T, Zeimet AG, Zeillinger R, Pils D, et al. Prognostic significance of L1CAM in ovarian cancer and its role in constitutive NF-κB activation. *Ann Oncol.* 2012;23(7):1795-802.
30. Altevogt P, Doberstein K, Fogel M. L1CAM in human cancer. *Int J Cancer.* 2016;138(7):1565-76.
31. Guo X, Xiong L, Zou L, Sun T, Zhang J, Li H, et al. L1 cell adhesion molecule overexpression in hepatocellular carcinoma associates with advanced tumor progression and poor patient survival. *Diagn Pathol.* 2012;7:96.
32. Fischer KR, Durrans A, Lee S, Sheng J, Li F, Wong ST, et al. Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. *Nature.* 2015;527(7579):472-6.