Observational Study

Medicine®

OPEN

Increased L1CAM (CD171) levels are associated with glioblastoma and metastatic brain tumors

Robin Wachowiak, MD[a,*], Matthias Krause, MD[b], Steffi Mayer, MD[a], Nicole Peukert[a], Anne Suttkus, PhD[a], Wolf C. Müller, MD[c], Martin Lacher, MD[a], Jürgen Meixensberger, MD[b], Ulf Nestler, MD[b]

Abstract

L1 cell adhesion molecule (L1CAM) is a member of the immunoglobulin-like cell-adhesion molecule family that was shown to be associated with a worse prognosis in several human cancers. L1 ectodomain shedding via vesicles or exosomes has been detected in extracellular fluids after cleavage from the cell surface by metalloproteases. We evaluated the presence of L1CAM in cyst fluid and tissue from glioblastomas or brain metastases.

The amount of L1CAM in cyst fluid of 9 glioblastomas and 11 brain metastases was assessed using enzyme-linked immunosorbent assay (ELISA). Corresponding tumor tissue slices were stained immunohistochemically for L1CAM. Cerebrospinal fluid of 20 non-tumor patients served as controls.

Mean levels of L1CAM in tumor cyst fluid were significantly higher in glioblastoma (6118 ± 4095 ng/mL) and metastasis patients (8001 ± 6535 ng/mL) than in CSF of control patients (714 ± 22 ng/mL). The immunohistochemical expression of L1CAM in corresponding tissue was significantly higher in metastases than in glioblastomas.

The present study demonstrates high levels of L1CAM in cyst fluid of glioblastoma and metastatic brain tumors. Soluble L1CAM may represent a motility promoting molecule in cancer progression, a co-factor for development of tumor cysts and a target for new treatment strategies.

Abbreviations: ADAM = a disintegrin and metalloproteinase, CSF = cerebrospinal fluid, ELISA = enzyme-linked immunosorbent assay, FFPE = formalin-fixed paraffin embedded, HRP = horseradish peroxidase, IRS = immunoreactive score, L1CAM = L1 cell adhesion molecule, SD = standard deviation, sL1CAM = soluble L1CAM, TBS = Tris-buffered saline, TGF-β = transforming growth factor-β, VEGF = vascular endothelial growth factor, WHO = World Health Organization.

Keywords: cyst fluid, glioblastoma, immunohistochemistry, L1 cell adhesion molecule, tumor markers

1. Introduction

Glioblastoma is one of the most common and most aggressive primary malignant brain tumors. Despite multimodal therapy, prognosis remains uniformly fatal with an expected survival of <15 months.[1] In glioblastoma patients, cystic tumors are encountered in 7% to 13% of cases.[2] However, the formation of tumor cysts is not only a feature of glioblastomas, but can also be found in astrocytomas, meningiomas, and especially in brain metastases. There are differing reports, whether the presence of a cystic compartment is associated with increased survival rate.[3–6] The cyst fluid inside these intratumoral compartments may be depending on its protein content, an important determinant for the growth potential of the surrounding tumor cells. Therefore, the analysis of the cyst content can enhance knowledge on pathophysiologic pathways of the underlying tumor, which may result in targeted treatment strategies.[7]

L1 is a 200 to 220 kDa multidomain type1 membrane glycoprotein of the immunoglobulin superfamily, which plays a central role during neural development and in regeneration of the adult nervous system.[8] L1 cell adhesion molecule (L1CAM) participates in myelination processes and promotes neural cell survival.[9] The extracellular domain of L1 can be cleaved regulatorily from the tumor cell surface in a process called shedding. The process of L1CAM ectodomain shedding, mediated by proteases such as ADAM 10 (a disintegrin and metalloproteinase), enhances cancer cell migration in various extracellular matrix components through autocrine or paracrine binding of integrins.[10]

Thus, L1CAM can also be detected as soluble form of L1CAM in the serum and ascites of patients with ovarian, uterine, or further cancers.[11–13]

L1CAM ectodomain shedding may also play a role in the development of brain tumor cysts. Therefore, the aim of the study was to examine the presence and concentration of L1CAM in tumor cyst fluid and solid tissue of patients with glioblastomas or brain metastases. Cerebrospinal fluid of non-tumorous patients with hydrocephalus or spinal stenosis served as controls.

2. Material and methods

2.1. Patients and clinical data

The study was approved by the Ethics committee of the University of Leipzig and informed consent of patients was obtained (reference number: 330-13-18112013). Nine patients with primary cystic glioblastoma and 11 patients with cystic

Editor: Jianxun Ding.

The authors of this work have no conflicts of interest to disclose.

[a] Department of Pediatric Surgery, [b] Department of Neurosurgery, [c] Department of Neuropathology, University Hospital Leipzig, Leipzig, Germany.

[*] Correspondence: Robin Wachowiak, Department of Pediatric Surgery, University Hospital Leipzig, Liebigstrasse 20 A, 04103 Leipzig, Germany (e-mail: robin.wachowiak@medizin.uni-leipzig.de).

Medicine (2018) 97:38(e12396)

Received: 12 June 2018 / Accepted: 23 August 2018

http://dx.doi.org/10.1097/MD.0000000000012396

metastasis were investigated for L1 levels in aspiration fluids from tumor cysts. Corresponding tissue samples from all patients were taken at the time of surgery and were evaluated for L1CAM expression by immunohistochemistry. Cerebrospinal fluid (CSF) from 10 patients with normal pressure hydrocephalus and 10 non-tumor bearing, healthy individuals who had undergone lumbar puncture, were taken as controls. CSF was collected either by lumbar or ventricular puncture. Brain tumor samples, obtained during open or stereotactic surgery, were routinely processed for histology and diagnosed according to the World Health Organization (WHO) classification of brain tumors. In all patients, cyst fluid was collected by intraoperative puncture of the tumor cyst.

2.2. Sample processing

Cyst fluid in each individual patient and tissue samples were collected during the same neurosurgical intervention. Cyst fluid samples were centrifuged at $1000 \times g$ for 5 minutes at 4 °C. Cell free supernatants were collected, separated in aliquots and stored frozen at –20 °C until use.[2]

2.3. L1CAM immunoassay

A commercially available enzyme-linked immunosorbent assay (ELISA) was used to measure the concentration of human L1CAM in the cyst fluids or CSF samples (Hoelzel Diagnostica, ELH-L1CAM-1, Cologne, Germany). ELISAs were run according to the manufacturer's protocol. In brief, anti-human-L1CAM antibody coated plates were incubated with fluid samples. After washing, the secondary antibody (biotinylated anti-human L1CAM) was added, followed by streptavidin-horseradish peroxidase (HRP) and tetramethylbenzidine. The reaction was then stopped with sulfuric acid. The plates were washed between each of the previous steps, except for the final one. ELISAs were run in duplicates and the 96-well plates were read at 450 nm on a Fluostar optima microplate reader (BMG, Labtech, Ortenberg, Germany). L1CAM concentrations were then determined using a log–log standard curve as detailed by the manufacturer.

2.4. L1CAM immunohistochemistry

Immunohistochemical staining was performed on 4-μm sections of formalin-fixed paraffin embedded (FFPE) tissue of 9 cystic glioblastomas and 11 cystic metastases from which cyst fluid had also been tested for presence of L1CAM by ELISA. After deparaffinization and rehydration in ethanol and Tris-buffered saline (TBS), tissues were incubated in a steamer with Target Retrieval Solution pH 9.0 (Dako, Hamburg, Germany). L1CAM was detected using a primary mouse monoclonal antibody clone UJ127 from Thermo Fisher Scientific (dilution 1:50, Waltham, MA). The Envision System HRP staining kit (Dako, Hamburg, Germany) was used to visualize the immunostaining. Sections were counterstained with Mayer haemalaun solution (Carl Roth, Karlsruhe, Germany). Finally, slides were covered with entellan (Merck, Darmstadt, Germany). Samples were analyzed under a light microscope (Zeiss, Jena, Germany) by 2 observers (UN, RW) that were blinded to the origin of the tissue. A semiquantitative histological scoring system, the immunoreactive score (IRS), was used according to Remmele and Stegner: intensity was defined as 0 = none, 1 = weak, 2 = moderate, and 3 = strong staining and multiplied with the percentage of positive cells including 0 = no cells, 1 = up to 10%, 2 = 10% to 50%, 3 = 51% to 80%, as well as 4 = >80% with the intensity of staining assessed first.[14] L1CAM expression was subsequently graduated in IRS Score 0 to 2 = negative (Fig. 1A and B), 3 to 4 = weakly positive, 6 to 8 = moderately positive, and 9 to 12 = strong (Fig. 1C and D).

2.5. Statistical analysis

Statistical analysis was performed using JMP 9 (SAS Institute, Cary, NC) and results were plotted by Prism 5 (GraphPad Software Inc., La Jolla, CA) software. All continuous data were nonparametric, thus a Mann–Whitney *U* test for 2-group comparison or a Kruskal–Wallis test with post hoc pairwise comparison for multiple-tests was applied. Regression analysis was performed to test the correlation between L1CAM expression as assessed by ELISA and histological staining. Significance level was set at $P < .05$. Data are presented as mean ± standard deviation (SD) if not indicated differently.

3. Results

3.1. Patient characteristics

In total, 9 patients with primary cystic glioblastoma and 11 patients with cystic metastasis (3 bronchogenic adenocarcinoma, 2 non-small cell lung cancer, 2 melanomas, 1 renal cell carcinoma, 1 ovary carcinoma, 1 colorectal carcinoma, and 1 breast cancer) as well as 20 controls were included in this study. Patient characteristics are summarized in Table 1.

3.2. L1CAM in cyst fluid

The mean amount of soluble L1CAM (sL1CAM) in aspiration fluids of glioblastoma cysts was 6118 ± 4095 ng/mL and in tumor cysts of metastases 8001 ± 6535 ng/mL. Although higher sL1CAM levels were noticed in brain metastases, the difference did not reach significance ($P = .7$; Fig. 2A). In cerebrospinal fluids of the controls, the level of sL1CAM (714 ± 22 ng/mL) was significantly lower as compared with tumor cyst fluids of glioblastomas and metastases ($P = .000$).

3.3. L1CAM expression in tissue of glioblastomas and brain metastases

According to the IRS scoring, mean L1 expression in glioblastoma specimen (3.6 ± 1.7) was significantly lower than in brain metastases (6.7 ± 3.9; $P = .04$ Fig. 2B). There was no significant correlation between L1CAM levels assessed by ELISA and IRS scoring system (Fig. 2C). ELISA levels and histologic score were analyzed by linear regression, meaning a higher concentration in the cyst fluid could also be found in tumors with weak L1CAM staining.

4. Discussion

In glioblastoma different studies have demonstrated strong L1 expression in tumor tissue. Tsuzuki et al[15] reported on mutation within the p53 gene or expression of TGF-β may upregulate L1 gene expression. Moreover, L1 expression results in a high migration rate of glioma cells. Likewise, it has been suggested that the cleaved L1 ectodomain supports the extensive and diffuse migratory behavior of glioblastoma within the brain tissue, therefore providing the basis of an autocrine model for glioblastoma invasion.[16]

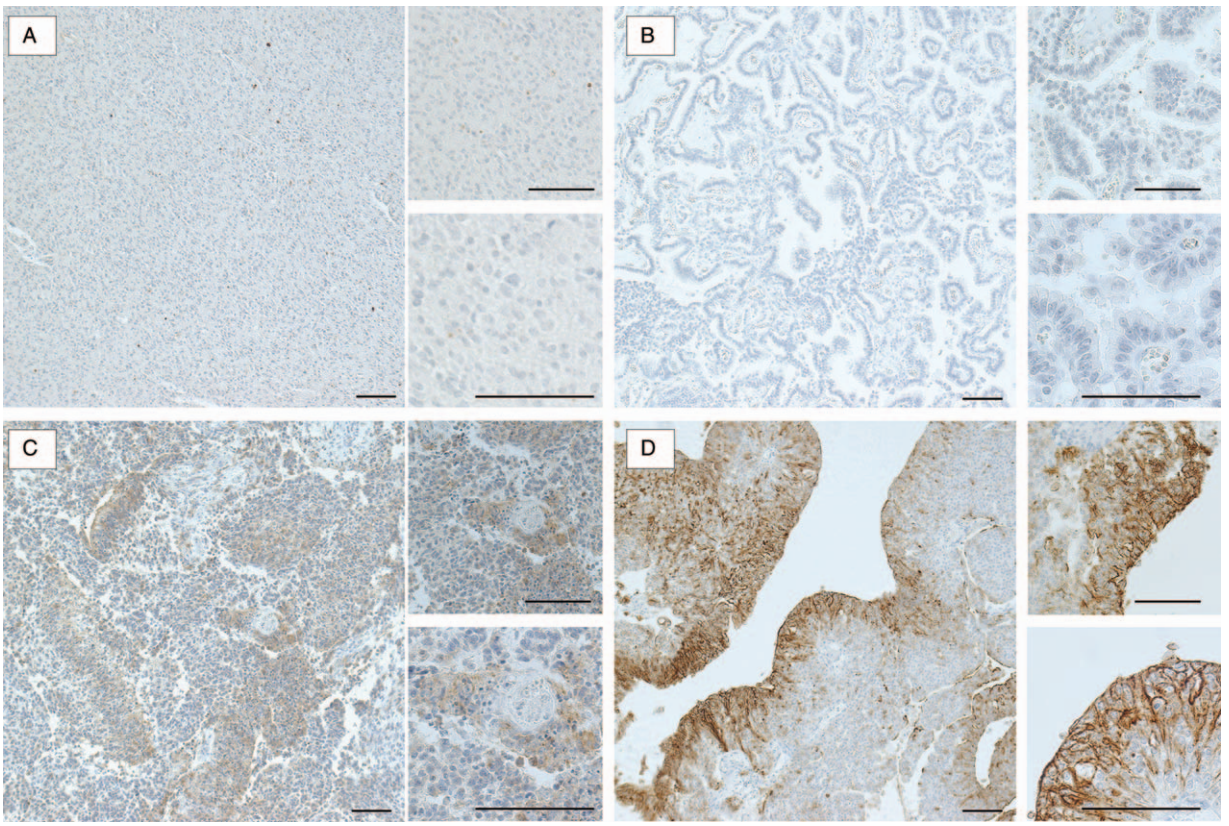

Figure 1. Immunohistochemical staining of L1CAM in tumor tissue of glioblastoma (A/C) and metastasis (B = papillary carcinoma/D = ovarian carcinoma). Immunoreactive scoring according to Remmele and Stegner was classified from weak (A, B) to strong (C, D) based on intensity of L1CAM staining and percentage of positive cells.[14] Scale bar 100 μm. L1CAM = L1 cell adhesion molecule.

In our study we detected high concentrations of soluble L1CAM in intratumoral cyst fluid of glioblastoma and by immunohistochemical staining of the protein in tumor tissue. This supports the hypothesis that intracystic proteins and the cyst fluid are synthesized by the surrounding tumor cells. Accordingly, the high concentrations of sL1CAM can be explained by cleavage of the L1CAM ectodomain from the surface of the tumor cells, as it was found in ascites of ovary cancer patients.[10] Based on a study of cell lysates and culture medium in human glioblastoma U87-MG cells, Zhao[17] suggested that soluble L1 may contribute to malignancy related cell migration and metastatic phenotype.

There have been only few reports about ELISA analysis in brain tumor cyst fluids. Several studies analyzed the fluid of glioblastoma cysts with regards to proteins such as vascular endothelial growth factor (VEGF)[18,19] or nutrient concentrations, (glutamate, lactate, and phosphate).[20]

To the best of our knowledge this is the first study which investigates L1CAM in cyst fluids of glioblastomas and brain metastases. We showed that cystic fluids of brain metastases contain an equal level of L1CAM compared with those of glioblastomas. Moreover, a significant higher expression of L1CAM in tissues of brain metastases was detected, even though they originate from different primary tumors and thus constitute of less homogenous tissue specimens than glioblastomas. In a number of primaries (including lung carcinoma, ovarian carcinoma, and colorectal cancer) earlier studies have described L1CAM expression.[11,21–24] The soluble ectodomain of L1CAM is functionally active and promotes cell migration as it was demonstrated in glioma cells.[16] Hypothetically, the high levels of L1CAM reflect increased cell motility and cell migration, potentially leading to worse outcome. In the present study, the number of patients was too small to perform an outcome analysis with reasonable statistic power due to the low number of patients with cystic tumors.

In contrast to the cerebrospinal fluid of controls, mean L1CAM concentrations were on average 8-fold increased in the cystic fluid

Table 1

Patients characteristics.

	Control group (n = 20)			Tumor group (n = 20)		
Variable	**Non-tumor patients (n = 10)**	**Hydrocephalus (n = 10)**	**Total**	**Metastasis (n = 11)**	**Glioblastoma (n = 9)**	**Total**
Age, y mean ± SD	55.6 ± 21.2	33.6 ± 33.4	44.62 ± 29.5	64.2 ± 10.86	67.7 ± 15.3	65.75 ± 12.8
Gender						
Male	6 (60%)	5 (50%)	11 (55%)	6 (54.5%)	6 (66.7%)	12 (60%)
Female	4 (40%)	5 (50%)	9 (45%)	5 (45.5%)	3 (33.3%)	8 (40%)

SD = standard deviation.

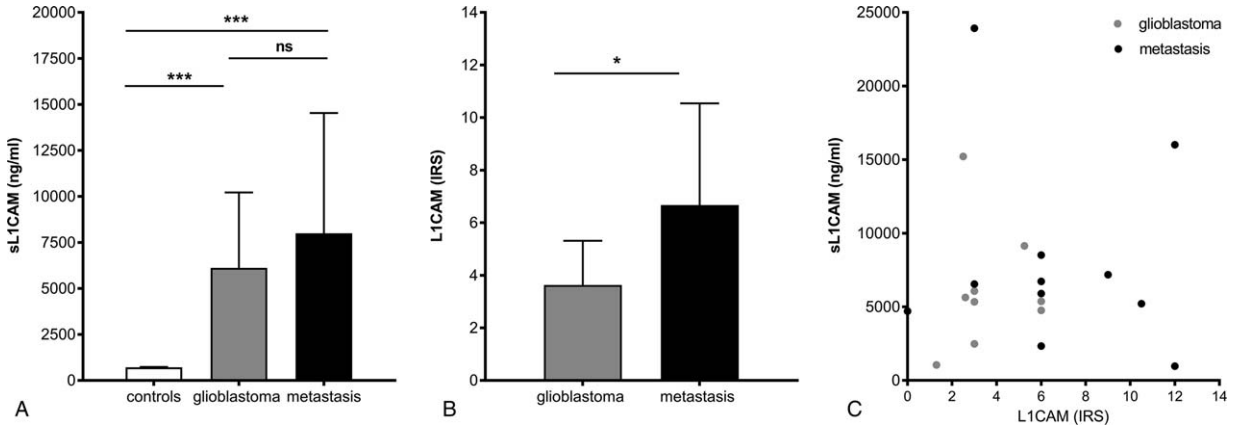

Figure 2. L1CAM expression in cyst fluid (ELISA) and solid tissue (histology) of glioblastoma or brain metastasis. L1CAM was significantly higher expressed in tumor cyst fluids of glioblastoma and brain metastasis as compared with control CSF ($P = .000$, A) but did not differ amongst each other ($P = .7$, A) although a significantly higher expression in metastasis than glioblastoma tissue was observed ($P = .04$, B). Accordingly, soluble and solid L1CAM expression did not correlate (C). Statistical analysis by Kruskal–Wallis test with post hoc pairwise comparison (A), Mann–Whitney *U* test (B) and linear regression (C); $P < .05^{*}$, $P < .01^{**}$, $P < .001^{***}$. CSF = cerebrospinal fluid, ELISA = enzyme-linked immunosorbent assay, L1CAM = L1 cell adhesion molecule.

of glioblastoma and 11-fold higher in brain metastases. This underlines the role of L1CAM as a promoting factor in malignant cerebral neoplasia.

We are aware of several limitations of the study, including the low number of cases and the inhomogeneous group of brain metastases, which did not allow further statements on the correlation between L1CAM detection and clinicopathological data. Therefore, larger studies are warranted to define the specific role of L1CAM in brain malignancies. However the determination of L1CAM concentrations in serum or CSF before and after neurosurgical intervention, as well as during subsequent adjuvant treatment, may allow the use of L1CAM as biomarker for brain tumors and for monitoring of treatment results, as it had been shown in ovarian cancer.[25]

5. Conclusion

In the present study, high concentrations of L1CAM were found in tumor cyst fluid of glioblastomas and brain metastases. This finding was confirmed by immunohistochemical detection of L1CAM expression in the corresponding tissue samples. In CSF of control patients, significantly lower ELISA levels of L1CAM were detected.

Our findings support the role of L1CAM shedding which is involved in progressive cancer cell migration and aggressive tumor growth. Further clinical studies are needed to elucidate the role of L1CAM in tumor pathogenesis, eventually opening a way to new treatment strategies in aggressive brain tumors.

Acknowledgments

The authors acknowledge support from the German Research Foundation (DFG) and Leipzig University within the program of Open Access Publishing.

Author contributions

Conceptualization: Robin Wachowiak, Matthias Krause, Ulf Nestler.
Data curation: Matthias Krause.
Formal analysis: Robin Wachowiak, Steffi Mayer, Ulf Nestler.
Investigation: Steffi Mayer, Nicole Peukert, Wolf C. Müller, Ulf Nestler.
Methodology: Robin Wachowiak, Matthias Krause, Nicole Peukert, Wolf C. Müller, Ulf Nestler.
Project administration: Robin Wachowiak, Martin Lacher, Jürgen Meixensberger, Ulf Nestler.
Supervision: Martin Lacher, Jürgen Meixensberger, Ulf Nestler.
Validation: Anne Suttkus, Wolf C. Müller, Jürgen Meixensberger.
Visualization: Anne Suttkus, Wolf C. Müller.
Writing – original draft: Robin Wachowiak, Matthias Krause, Ulf Nestler.
Writing – review & editing: Robin Wachowiak, Steffi Mayer, Anne Suttkus, Wolf C. Müller, Martin Lacher, Jürgen Meixensberger.

Robin Wachowiak: 0000-0003-2701-8692.

References

[1] Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987–96.
[2] Hoelscher M, Richter N, Melle C, et al. SELDI-TOF analysis of glioblastoma cyst fluid is an approach for assessing cellular protein expression. Neurol Res 2013;35:993–1001.
[3] Kaur G, Bloch O, Jian BJ, et al. A critical evaluation of cystic features in primary glioblastoma as a prognostic factor for survival. J Neurosurg 2011;115:754–9.
[4] Maldaun MV, Suki D, Lang FF, et al. Cystic glioblastoma multiforme: survival outcomes in 22 cases. J Neurosurg 2004;100:61–7.
[5] Sarmiento JM, Nuno M, Ortega A, et al. Cystic glioblastoma: an evaluation of IDH1 status and prognosis. Neurosurgery 2014;74:71–5. discussion 75-6.
[6] Utsuki S, Oka H, Suzuki S, et al. Pathological and clinical features of cystic and noncystic glioblastomas. Brain Tumor Pathol 2006;23: 29–34.
[7] Westphal M, Nausch H, Herrmann HD. Cyst fluids of malignant human brain tumors contain substances that stimulate the growth of cultured human gliomas of various histological type. Neurosurgery 1989;25:196–201.
[8] Moos M, Tacke R, Scherer H, et al. Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. Nature 1988;334:701–3.
[9] Conacci-Sorrell M, Kaplan A, Raveh S, et al. The shed ectodomain of Nr-CAM stimulates cell proliferation and motility, and confers cell transformation. Cancer Res 2005;65:11605–12.
[10] Fogel M, Gutwein P, Mechtersheimer S, et al. L1 expression as a predictor of progression and survival in patients with uterine and ovarian carcinomas. Lancet 2003;362:869–75.

[11] Bondong S, Kiefel H, Hielscher T, et al. Prognostic significance of L1CAM in ovarian cancer and its role in constitutive NF-kappaB activation. Ann Oncol 2012;23:1795–802.
[12] Tangen IL, Kopperud RK, Visser NC, et al. Expression of L1CAM in curettage or high L1CAM level in preoperative blood samples predicts lymph node metastases and poor outcome in endometrial cancer patients. Br J Cancer 2017;117:840–7.
[13] Zander H, Rawnaq T, von Wedemeyer M, et al. Circulating levels of cell adhesion molecule L1 as a prognostic marker in gastrointestinal stromal tumor patients. BMC Cancer 2011;11:1891–7.
[14] Remmele W, Stegner HE. [Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue]. Pathologe 1987;8:138–40.
[15] Tsuzuki T, Izumoto S, Ohnishi T, et al. Neural cell adhesion molecule L1 in gliomas: correlation with TGF-beta and p53. J Clin Pathol 1998; 51:13–7.
[16] Yang M, Adla S, Temburni MK, et al. Stimulation of glioma cell motility by expression, proteolysis, and release of the L1 neural cell recognition molecule. Cancer Cell Int 2009;9:27.
[17] Zhao W. Comparison of L1 expression and secretion in glioblastoma and neuroblastoma cells. Oncol Lett 2012;4:812–6.
[18] Stockhammer G, Obwegeser A, Kostron H, et al. Vascular endothelial growth factor (VEGF) is elevated in brain tumor cysts and correlates with tumor progression. Acta Neuropathol 2000;100:101–5.
[19] Weindel K, Moringlane JR, Marme D, et al. Detection and quantification of vascular endothelial growth factor/vascular permeability factor in brain tumor tissue and cyst fluid: the key to angiogenesis? Neurosurgery 1994;35:439–48. discussion 448–439.
[20] Dahlberg D, Struys EA, Jansen EE, et al. Cyst fluid from cystic, malignant brain tumors: a reservoir of nutrients, including growth factor-Like nutrients, for tumor cells. Neurosurgery 2017;80:917–24.
[21] Hai J, Zhu CQ, Bandarchi B, et al. L1 cell adhesion molecule promotes tumorigenicity and metastatic potential in non-small cell lung cancer. Clin Cancer Res 2012;18:1914–24.
[22] Kaifi JT, Reichelt U, Quaas A, et al. L1 is associated with micrometastatic spread and poor outcome in colorectal cancer. Mod Pathol 2007;20: 1183–90.
[23] Miyahara R, Tanaka F, Nakagawa T, et al. Expression of neural cell adhesion molecules (polysialylated form of neural cell adhesion molecule and L1-cell adhesion molecule) on resected small cell lung cancer specimens: in relation to proliferation state. J Surg Oncol 2001;77: 49–54.
[24] Tischler V, Pfeifer M, Hausladen S, et al. L1CAM protein expression is associated with poor prognosis in non-small cell lung cancer. Mol Cancer 2011;10:127.
[25] Aktas B, Kasimir-Bauer S, Wimberger P, et al. Utility of mesothelin, L1CAM and Afamin as biomarkers in primary ovarian cancer. Anticancer Res 2013;33:329–36.