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Clinicopathologic significance of LAIR-1 expression in hepatocellular carcinoma

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ABSTRACT

Aim: Leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1) is an immune inhibitory receptor which is expressed within most types of hematopoietic cells and negatively regulates immune responses. Recently, we found LAIR-1 expression to be present within tumors of nonhematopoietic lineages. However, the roles of LAIR-1 in hepatocellular carcinoma (HCC) have yet to be examined. The purpose of this study was to investigate the expression of LAIR-1 in HCC tissue and assess its clinical significance at this site.

Materials and methods: Expression levels of LAIR-1 within HCC samples collected from 90 patients and compared with that of slides of normal liver tissue collected from 9 non-HCC patients were measured by immunohistochemistry using

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tissue microarrays. A semiquantitative score was assigned, as was based on staining intensity and percent of positive cells and a Spearman Rank correlation test was used to assess any potential significant correlations between LAIR-1 expression and clinicopathological factors. Overall survival analysis was performed using the Kaplan-Meier and Log Rank statistical test.

Results: LAIR-1 expression was detected in cancer tissue and adjacent tumor tissue, but not in normal liver tissue. The percent of LAIR-1-positive expression in cancer tissue of HCC samples was 97.78% (88/90) while that in adjacent tumor tissue was 96.67% (87/90). Significantly greater expression levels of LAIR-1 were obtained from cancer tissue ($\text{Mean} \pm \text{SD} = 5.722 \pm 2.145$) than that in adjacent tumor tissue (4.141 ± 1.486). In addition, LAIR-1 expression was found to be significantly correlated with pathological grade of HCC, T stage, and age. Expression levels of LAIR-1 were related with worse overall survival rates of HCC patients, especially in HCC patients with hepatic cirrhosis.

Conclusion: Results of this study show that LAIR-1 is expressed in HCC tissues and that high levels of LAIR-1 expression are associated with the poor cancer differentiation. In addition, overexpression of LAIR-1 was significantly associated with worse overall survival in the patients with HCC. These data suggest that LAIR-1 may be an independent predictor for clinical outcomes in patients with HCC.

Introduction

Worldwide, hepatocellular carcinoma (HCC) currently represents the fifth most commonly occurring solid tumor mass and the third leading cause of cancer-related deaths.[1,2] In spite of remarkable progress in the treatment and understanding of HCC, the molecular mechanisms underlying HCC carcinogenesis remain to be elucidated.[3,4] Most HCC patients are considered incurable as a result of extensive heterogeneity in clinical presentations and tumor biology, which complicates the classification and procedures for therapy.[5,6] While survival of patients with HCC has improved with advances in surgical techniques,[7,8] in the late stages of this disease, lymph node metastasis or distant metastasis are present, rendering therapies including surgery, chemotherapy, and Chinese medicine treatment relatively ineffective. Therefore, a better understanding of the molecular pathogenesis of HCC is crucial for advancing the procedures necessary for an early diagnosis and treatment of this disease.

Leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1), which belongs to the immunoglobulin superfamily, also known as CD305, is an inhibitory collagen receptor which carries immunoreceptor tyrosine-based inhibition motifs in their cytoplasmic tail.[9] LAIR-1 is widely expressed in most immune cells, including natural killer cells, T cells, B cells, monocytes, and $CD34^+$ hematopoietic progenitor cells.[10-12] Results from previous work within our laboratory have revealed that LAIR-1 is also expressed in epithelial ovarian cancer cells and is involved in cell proliferation and invasion.[13] However, the issue of LAIR-1 expression and potential roles in tumors of nonhematopoietic lineage has received little, if any, attention. In specific, with regard to the present study, there is no information available regarding the expression of LAIR-1 in HCC.

In order to address these issues, here we examined whether expression of LAIR-1 would be present in HCC tissue and surveyed some of the potential clinical significance of LAIR-1 expression in HCC. To accomplish this goal, 90 samples of tumor tissue and adjacent tumor tissue were

collected from HCC patients and analyzed using tissue microarrays. These samples were compared with that of immunohistochemistry slides from that of 9 samples of normal liver tissue as collected from non-HCC patients. In this study, we also examined whether increased tissue expression levels of LAIR-1 were associated with worse clinical outcomes in HCC patients.

Materials and methods

Study population and data collection

Liver samples from a total of 90 HCC patients were obtained for this cohort study, which included 46 existent and 44 deceased patients. This study consisted of 77 men and 13 women with ages ranging from 1 to 72 years. As a control for comparison, liver samples from a total of 9 non-HCC patients were included. This study was approved by our Institutional Review Board and all subjects provided voluntary informed consent to participate in the study. Tumor diagnosis was performed by expert pathologists.

The HCC tissue microarrays (No: HLiv-HCC180Sur-01, Shanghai Outdo Biotech Co., Ltd.) were performed on samples obtained from 90 patients who underwent curative surgery for HCC. A cancer tissue sample and an adjacent tumor tissue sample were obtained from each patient resulting in a total of 180 samples from the 90 patients. The samples were obtained over the period from August 2006 to September 2010, and all patients were followed up in August 2012. Clinicopathological data were collected through assessment of medical records of these surgically treated HCC patients and included sex, age, tumor size, lymph node metastasis, distant metastasis, World Health Organization pathological grades, T stage, and clinical tumor, node, metastasis (TNM) stage. Eligibility for this study consisted of patients who were histologically diagnosed with HCC and previously received primary surgery.

Immunohistochemistry slices of liver tissues from 9 cases of non-HCC patients from the First People's Hospital of Changzhou served as a control for comparison with that of the HCC patients. These samples consisted of liver tissue obtained from patients with the following conditions: 5 adjacent hepatic hemangioma, 1 hepatic traumatism, 2 cholecystitis, and 1 gallbladder carcinoma.

Immunohistochemical staining

All specimens were fixed in 10% neutral buffered formalin and dehydrated through an ethanol gradient (30%, 50%, 70%, 80%, 95%, and 100% ethanol). Samples were then hyalinized in xylene and embedded in conventional liquid paraffin for subsequent immunohistochemical staining. All standard IHC reagents were provided by the Department of Histology and Embryology (Binzhou Medical University, China). After dewaxing, rehydration, and antigen retrieval according to standard operating procedures, sections were incubated overnight at 4 °C with the monoclonal antibody mouse antihuman LAIR-1 [lc12] (1:100; ab14826; Abcam, Cambridge, MA) as the primary antibody. Sections were washed 5 times with phosphate-buffered saline prior to incubation with the secondary antibody, goat antimouse (1:100) at 37 °C for 30 min. Antigen visualization was performed by diaminobenzidine (DAB-0031/1031kit, Maixin, Biological, Fuzhou, China) staining, followed by counterstaining with hematoxylin.

Semiquantitative analysis

All slides were independently evaluated by two pathologists blinded as to the clinical and histopathological data regarding the samples. Based on staining intensity and percent of positive cells, a semiquantitative score was generated as determined by evaluating three different areas

within each section. Intensity of staining was classified on a scale of 0-3+ (0: no staining, 1+: weak staining, 2+: moderate staining, and 3+: strong staining). The percent of positively stained cells was scored by cell counts on a scale of 0-4 (0: 0%, 1: 1%-25%, 2: 26%-50%, 3: 51%-75%, and 4: 76%-100%). A total evaluation assessment value was derived by multiplying intensity scores by cell positivity scores. Total values of ≤ 6 indicate low expressions of LAIR-1 and >6 indicate high expressions of LAIR-1.

Statistical analysis

Potential associations between clinicopathological characteristics of the HCC patients and LAIR-1 expression were analyzed using the Spearman Rank correlation. The Kaplan-Meier method and Log Rank statistical test were used to assess differences in survival rates. Differences in expression levels of LAIR-1 between tumor tissue and adjacent tumor tissue were analyzed using the Wilcoxon matched-pairs signed ranks test. Sex, tumor size, lymph node metastasis, and distant metastasis were classified as categorical variables; all others were considered as continuous variables. Statistical analyses were performed using the SPSS 17.0 software. A value of $P < 0.05$ was required for results to be considered statistically significant.

Results

Expression levels of LAIR-1 in HCC tissue

The results of IHC revealed that the expression of LAIR-1 was mainly concentrated within the cytoplasm of carcinoma cells and adjacent tumor tissue of HCC samples, but was not detected in normal liver tissue (Fig 1). Results of the immunohistochemical assay demonstrated that the percent of LAIR-1-positive expression in cancer tissue of HCC samples was 97.78% (88/90) while that in adjacent tumor tissue was 96.67% (87/90). The total score for LAIR-1 expression in cancer tissue of 5.722 ± 2.145 (Mean + SD) was significantly greater than that of the 4.141 ± 1.486 from adjacent tumor tissue (Figs 1B-F and 2A; $P < 0.001$). We also compared differences in expression levels of LAIR-1 in cancer tissue and that of in adjacent tumor tissue in patients with or without hepatic cirrhosis, respectively. Results from this analysis revealed that total scores of LAIR-1 expression in adjacent tumor tissue with hepatic cirrhosis (4.193 ± 1.460) was slightly, but not statistically, greater than that in adjacent tumor tissue without hepatic cirrhosis (4.063 ± 1.501; Figs 1B, C and 2B). There is no statistic difference of LAIR-1 expression levels in cancer tissue between patients with (6.455 ± 1.819) or without hepatic cirrhosis (5.663 ± 2.084) (Fig 2C). The total score for LAIR-1 expression in cancer tissue of 6.455 ± 1.819 was significantly higher than that of the 4.193 ± 1.460 from adjacent tumor tissue in patients with hepatic cirrhosis. LAIR-1 expression in cancer tissue (5.663 ± 2.084) was significantly greater than adjacent tumor tissue (4.063 ± 1.501) in patients without hepatic cirrhosis (Fig 2D, E; $P < 0.001$).

Correlations between LAIR-1 expression and clinicopathological parameters

To further examine the role of LAIR-1 in HCC progression, Spearman Rank correlations were performed between data on related clinicopathological parameters and expression levels of LAIR-1 in HCC. The results of this analysis showed that increased expressions of LAIR-1 in HCC were negatively related to patient's age ($r_s = -0.217$, $P = 0.041$; Fig 3A), positively correlated with T stage ($r_s = 0.227$, $P = 0.032$; Fig 3B), and the pathological grade of HCC ($r_s = 0.204$, $P = 0.01$; Fig 3C). No statistically significant correlations were obtained between LAIR-1 expression and other clinicopathological parameters including sex, tumor size, lymph node metastasis, distant metastasis, and clinical TNM stage. The clinicopathological parameters of the 90 study participants and expressions of LAIR-1 for selected study variables are presented in Table 1.

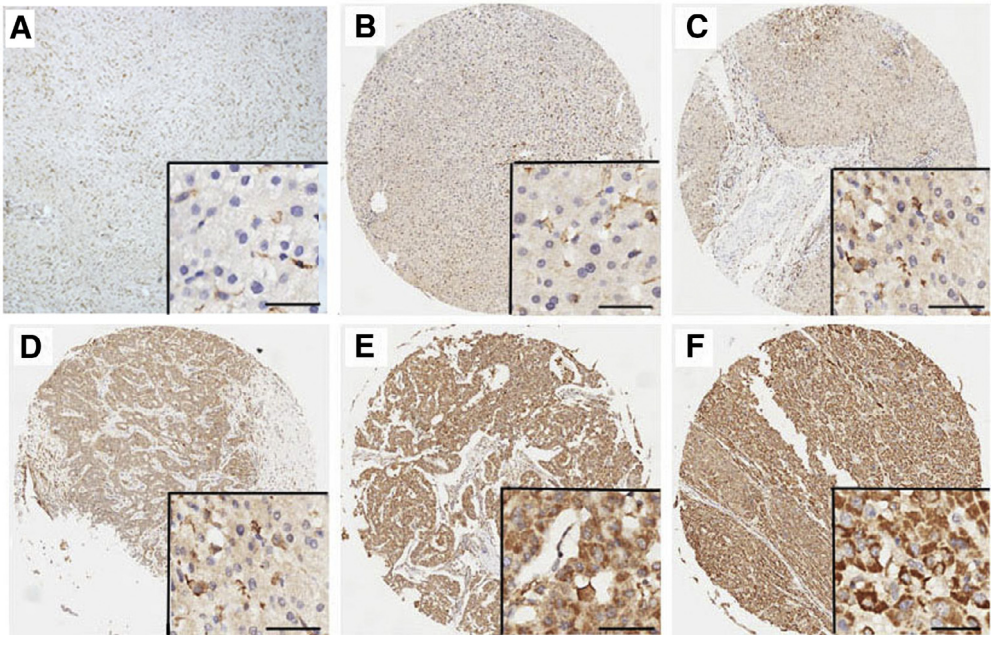

Fig. 1. Expression levels of LAIR-1 in HCC cancer tissue, adjacent tumor tissue and normal liver tissue. (A) Normal liver tissue. (B) Adjacent tumor tissue without hepatic cirrhosis. (C) Adjacent tumor tissue with hepatic cirrhosis. (D) Grade I. (E) Grade II. (F) Grade III original magnification × 100. Scale bar = 20 μM.

Table 1
Correlations between LAIR-1 expression in cancer tissue and clinicopathological parameters of patients with HCC ($N = 90$).

Variable		No. of patients	LAIR-1		*P* value
			High expression (%)	Low expression (%)	
Sex	Male	77	20	57	0.665
	Female	13	3	13	
Age	≤60	68	23	45	**0.041**
	>60	21	1	20	
Pathological grade	I	3	0	3	
	I-II	7	0	7	
	II	44	12	32	**0.010**
	II-III	21	6	15	
	III	14	5	9	
Tumor size	≤5 cm	38	5	33	0.055
	>5 cm	51	17	34	
T stage	T_1	11	2	9	
	T_2	33	7	26	**0.032**
	T_3	42	12	30	
	T_4	3	2	1	
Lymph node metastasis	N_0	84	22	62	0.932
	N_1	1	0	1	
Distant metastasis	M_0	87	22	65	0.934
	M_1	1	0	1	
TNM stage	I	11	2	9	
	II	32	6	26	0.072
	III	41	13	28	
	IV	2	0	2	

Bold indicates that the *P* value is statistically significant.

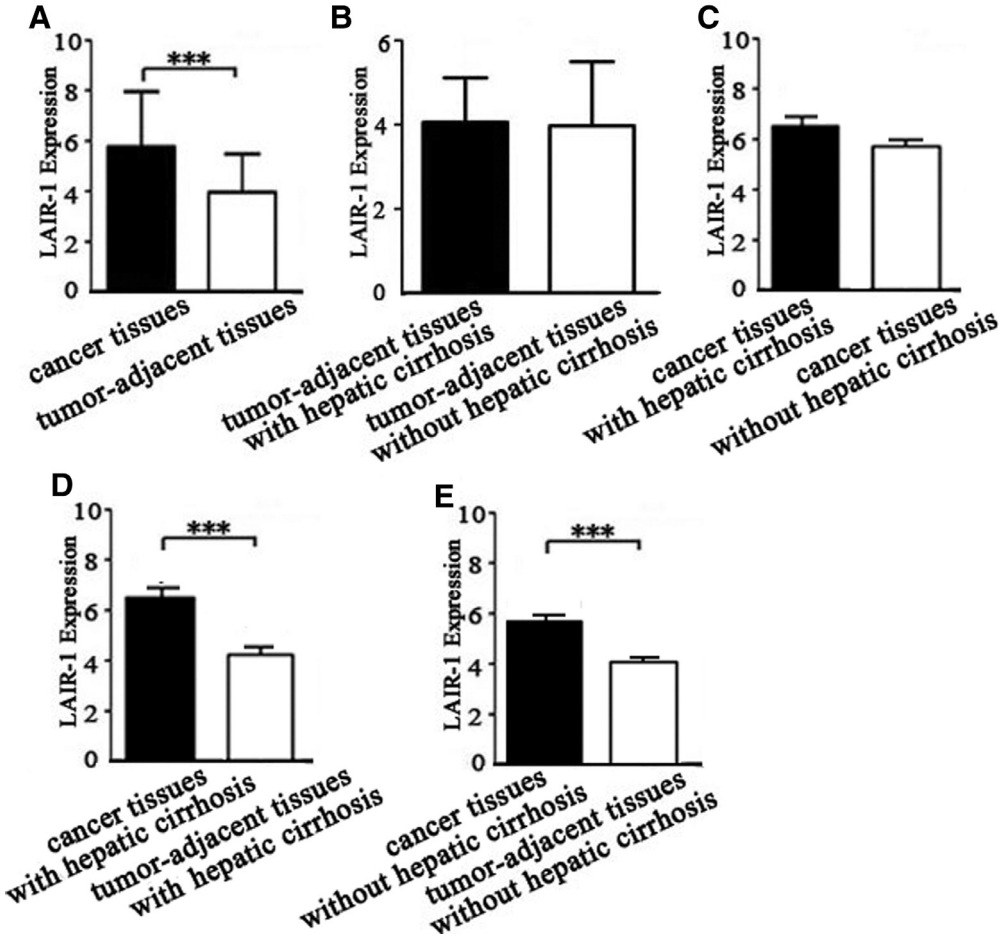

Fig. 2. Statistical analysis of LAIR-1 expression levels between HCC cancer tissue and adjacent tumor tissue. (***$P < 0.001$).
(A) LAIR-1 expression levels in cancer tissue and adjacent tumor tissue in all patients.
(B) LAIR-1 expression levels in adjacent tumor tissue between patients with or without hepatic cirrhosis.
(C) LAIR-1 expression levels in cancer tissue and adjacent tumor tissue in patients with hepatic cirrhosis.
(D) LAIR-1 expression levels in cancer tissue and adjacent tumor tissue in patients without hepatic cirrhosis.

Survival analysis

In Kaplan-Meier analysis, LAIR-1 expression, large tumor size, late clinical T stage, and late clinical TNM stage were found to be significant risk factors in patients who underwent HCC resection. It is noteworthy that high levels of LAIR-1 expression were significantly associated with worse overall survival rates than those with low expression levels within the cancer tissue ($P < 0.05$), especially in HCC patients with hepatic cirrhosis (Fig 4A, B). However, expression levels of LAIR-1 were not associated with any differences in overall survival in HCC patients without hepatic cirrhosis (Fig 4C).

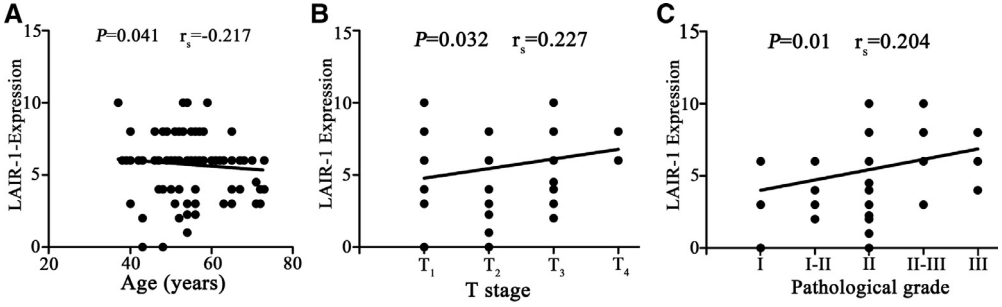

Fig. 3. Correlations between LAIR-1 expression in HCC tumor tissue and pathological grade, T stage and age.
(A) Correlation between LAIR-1 expression in HCC tumor tissue and age. (B) Correlation between LAIR-1 expression in HCC tumor tissue and T stage. (C) Correlation between LAIR-1 expression in HCC tumor tissue and pathological grade.

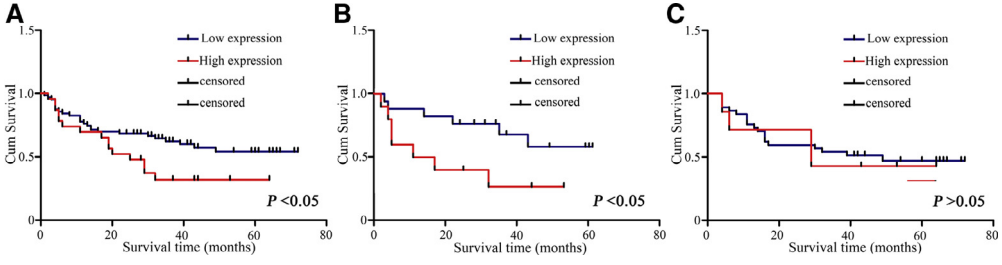

Fig. 4. Overall survival of HCC patients with high and low expressions of LAIR-1 as determined with Kaplan-Meier survival curves.
(A) Expression of LAIR-1 in cancer tissue of HCC patients. (B) Expression of LAIR-1 in cancer tissue of HCC patients with hepatic cirrhosis. (C) Expression of LAIR-1 in cancer tissue of HCC patients without hepatic cirrhosis.

Discussion

Since its discovery, LAIR-1 has been reported to be mainly expressed in hamatopoietic cells and to function as an inhibitory receptor. LAIR-1 can bind to the conserved Gly-Pro-Hyp repeats of collagens and negatively regulate immune responses and cell differentiation.[14,15] As a ligand for LAIR-1, collagen is also extensively expressed in the extracellular matrix.[9,14] Upon engagement of LAIR-1, the tyrosines within its immunoreceptor tyrosine-based inhibition motifs undergo phosphorylation to recruit phosphatases SHP-1, SHP-2, and C-terminal Src kinse (Csk), which negatively regulates immune responses and cell differentiation.[9,16] It has been reported that many tumor types overexpress collagens which correlate with unfavorable clinical outcomes that can modulate immune cell function through the inhibitory collagen receptor LAIR-1.[17] However, the roles of LAIR-1 in tumors of nonhematopoietic lineage have yet to be investigated, which raise the question of whether any relationships exist between LAIR-1 and other tumors. Previous work within our laboratory has revealed that soluble levels of LAIR-1 in sera from tumor patients were significantly increased as compared with that in healthy individuals and the expression of LAIR-1 on $CD3^+CD4^+$ T or $CD3^+CD8^+$T cells within the peripheral blood of cervical cancer and endometrial carcinoma patients was significantly greater than that in patients with hysteromyom or precancerous lesions.[18] A subsequent follow-up study showed that LAIR-1 was expressed in epithelial ovarian cancer cells and was involved in cell proliferation and invasion.[13] In our current study, we now show that LAIR-1 is expressed in HCC tissues and expression levels of LAIR-1 in carcinoma tissue were significantly greater than that obtained from adjacent tumor tissue. In contrast, LAIR-1 expression was not detected in normal liver tissue. These data suggest LAIR-1 may be a potential biomarker for patients with HCC.

There is also evidence indicating that LAIR-1 is closely related to leukemia. For example, LAIR-1 has been reported to inhibit the proliferation of human primary leukemia and myeloid leukemia cell lines.[19,20] LAIR-1 expression has also been shown to be at significantly higher levels in primary human acute myeloid leukemia bone marrow and peripheral blood mononuclear cells than that observed in normal counterparts; and these levels negatively correlate with the overall survival of acute myeloid leukemia patients.[21] Results from several studies have indicated that the expression levels of LAIR-1 in chronic lymphocytic leukemia (CLL) are related to the stage of the disease. Moreover, LAIR-1 is absent in high-risk CLL and differentially expressed in intermediate-risk and low-risk CLL patients; and the intensity of its expression, which is always significantly lower than in healthy donors, correlates with disease stage and progression.[22,23] In this study, we examined expression levels of LAIR-1 in HCC progression as related to clinicopathological parameters. The results of this analysis showed that increased levels of LAIR-1 expression in HCC were negatively related to patient's age and positively correlated with T stage. It is noteworthy that overexpression of LAIR-1 was significantly associated with worse overall survival in the patients with HCC, a phenomenon that was more significant in HCC patients with hepatic cirrhosis. These results suggest that LAIR-1 may be an excellent predictor for poor clinical outcomes in HCC patients.

Findings from previous studies have demonstrated that higher expression levels of LAIR-1 are related to a less-differentiated phenotype in several types of hamatopoietic cells, such as neutrophil, hematopoietic progenitors, and megakaryocytes.[11,12,24] In this study, we found that expression levels of LAIR-1 were positively correlated to the pathological grade of HCC, which indicates that the higher the LAIR-1 expression, the worse the cancer differentiation.

In conclusion, to the best of our knowledge, the findings of this study are the first to demonstrate that LAIR-1 is expressed in HCC tissue and that high expression levels of LAIR-1 are associated with poor differentiation of HCC cells. We also reveal that LAIR-1 is significantly associated with worse clinical outcomes in HCC patients, which provides a new potential option for the development of prognostic factors. Our findings suggest that LAIR-1 has a relevant impact on HCC progression and may be helpful for a better understanding of the molecular pathogenesis of cancer. The precise mechanisms of LAIR-1 that influence biological functions of carcinoma cells deserve further investigation.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.currproblcancer.2018.04.005.

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