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Oncolytic adenovirus targeting LASP-1 inhibited renal cell cancer progression

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Abstract: Recent studies suggested that LIM and SH3 protein 1 (LASP-1) is a promising therapeutic target for renal cell cancer (RCC). This study aimed to explore the role of LASP-1 in RCC. For this purpose, LASP-1 expression in RCC tissues was analyzed by immunohistochemistry and Western blot analysis. Cell proliferation, migration, invasion, and gene expression were detected by CCK-8 assay, Transwell assay, and Western blot analysis. The results showed that LASP-1 was highly expressed in RCC, and its expression level,t was positively correlated with lymph node metastasis and tumor, nodes, and metastases (TNM) stage. The knockdown of LASP-1 expression significantly inhibited the proliferation of RCC cells, increased the apoptosis rate, and inhibited RCC cell invasion and migration by inhibiting epithelial–mesenchymal transition. We conclude that LASP-1 promotes RCC progression and metastasis and is a promising therapeutic target for RCC.

Introduction

Renal cell cancer (RCC) is a common urological cancer worldwide (Leibovich *et al.*, 2010; Kammerer-Jacquet *et al.*, 2018). Due to recurrence or metastasis, the mortality of RCC remains as high as 40% (Bex *et al.*, 2012; Simonaggio *et al.*, 2018). Therefore, it is important to develop new treatments for RCC.

LIM and SH3 protein 1 (LASP1) was first identified as a scaffold protein that regulated cytoskeleton dynamics and cell migration in breast cancer (Grunewald *et al.*, 2007a). Later studies showed that LASP-1 was overexpressed in a variety of other cancers (Salvi *et al.*, 2015; Zhao *et al.*, 2015; Grunewald *et al.*, 2007b). In addition, the high LASP-1 expression level was correlated with poor outcomes in cancer patients, including RCC patients (Yang *et al.*, 2014). Recent studies have shown that LASP1 promotes cancer cell proliferation, migration, and invasion via the interaction with the cytoskeleton and the regulation of signaling pathways such as PI3K/Akt in various tumors (Butt and Raman, 2018).

Up to now, few studies have examined the function of LASP-1 in RCC. Therefore, in this study, we aimed to investigate the expression level and functional role of LASP-1 in RCC. We analyzed the correlation of LASP-1 expression to the clinicopathological features of RCC. Next, we constructed oncolytic adenovirus to knockdown LASP-1 to develop a new strategy for RCC treatment.

Materials and Methods

Immunohistochemical staining

RCC tissues were collected from RCC patients who visited the Department of Urology, The First Affiliated Hospital of Soochow University, and were processed following standard protocols for immunohistochemical staining. The study was approved by the Ethics Committee of The First Affiliated Hospital of Soochow University, and all participants signed informed consent. Briefly, the sections were incubated with a LASP-1 antibody (1:500 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and then the sections were stained using avidin-biotin ABC kit (Vector Laboratories, Burlingame, CA, USA). For negative control, the primary antibody was replaced by saline. Five fields were randomly selected from each sample, and immunohistochemical staining was assessed based on staining intensity. The staining intensity was graded by a four-point scale: –, no staining; +, light yellow; ++, brown; +++, dark brown. Only samples with ++ and +++ were judged as positive staining, and the percentage of samples with positive standing was calculated.

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Cell culture
Human RCC cell line 786-0 was purchased from American Type Culture Collection (Rockville, MD, USA) and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS).

Construction of recombinant adenovirus
Oncolytic adenovirus ZD55-LASP-1 was constructed by homologous recombination between pZD55-LASP-1 and adenovirus packaging plasmid pBHGE3 (Microbix Biosystems, Toronto, Canada), and recombinant adenovirus was verified by PCR.

Cell viability assay
The cells were plated in 96-well plates, and 24 h later were infected with adenovirus. Next, the cells were collected, and cell viability was analyzed by CCK-8 assay using a Cell counting kit (Dojindo Molecular Technologies, Gaithersburg, MD, USA).

Migration and invasion assay
Cell migration and invasion assays were performed in chamber plates (pore size: 8 μm) following standard protocols. For invasion assay, Matrigel was diluted in serum-free medium, and then the upper chambers were coated with Matrigel overnight. Next, the cells were seeded in serum-free medium in the upper chamber and cultured at 37°C. After 24 h, the cells in the upper chamber were removed, and the cells that crossed the membrane were fixed and stained with trypan blue. Migration assay was performed similarly to the invasion assay except that the upper chambers were not coated with Matrigel overnight.

Western blot analysis
Cells were washed and lysed in lysis buffer on ice. Protein concentrations in lysates were determined by bicinchoninic acid assay, and equal amounts of proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Next, the proteins were transferred onto nitrocellulose membranes, which were incubated with primary antibodies for LASP-1, vimentin, E-cadherin, N-cadherin, E1A, and β-actin (1:1000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and peroxidase-conjugated secondary antibody (1:2000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The blots were developed with Pierce™ Fast Western Blot Kit (Pierce, Rockford, IL, USA) and analyzed with Image.plus5.1 software on an image analyzer.

Xenograft tumor model in nude mice
Animal procedures were approved by the Animal Care and Use Committee of Xuzhou Medical College. BALB/c nude mice (male, 4–5 weeks old) were obtained from the Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China). $2x10^6$ 786-0 cells were subcutaneously injected into the right flank of mice. When tumor volume reached 100 mm^3, a total of 32 mice were randomly divided into four groups (8 mice in each group) and received intratumoral injection of ZD55-LASP-1, ZD55-EGFP, Ad-LASP-1, or saline (100 μL), respectively, every other day three times. Tumor volume was measured with the formula: V (mm^3) = length x $width^2/2$.

TUNEL assay
Apoptotic cells in tissues were detected by using *in situ* apoptosis detection kit (Roche, Indianapolis, IN, USA) following the manufacture's protocol. Six fields were randomly selected from each slide, and positive-stained cells were counted to calculate the apoptotic rate.

Statistical analysis
Statistical analysis was performed by one-way analysis of the variance (ANOVA) followed by the Newman-Keuls test. $P < 0.05$ was considered significant.

Results

LASP-1 expression is correlated with clinicopathological features of RCC
Immunohistochemistry analysis of LASP-1 expression in RCC tissues showed that positive LASP-1 staining was 92.68% (76/82) in 82 cases of renal carcinoma and 26.83% (22/82) in 82 cases of adjacent normal tissue. LASP-1 was predominantly stained in the nuclei of RCC cells (Figs. 1 and 2A). Increased LASP-1 expression was positively correlated with TNM stage ($Z = -3.473$, $P = 0.001$; $r = 0.386$) (Fig. 2B, Tab. 1). In addition, LASP-1 staining was stronger in renal carcinoma with lymph node metastasis (lymphatic metastasis group) than in RCC without lymph node metastasis (non-lymphatic metastasis group) ($Z = -6.401$, $P < 0.001$) (Figs. 1 and 2C, Tab. 1). Moreover, high expression of LASP-1 was positively correlated with tissue type, TNM stage, and lymph node metastasis ($P < 0.05$), but not with tumor size or Furhman grade ($P > 0.05$).

Furthermore, Western blot analysis of RCC tissues showed that the LASP-1 protein level in the lymphatic metastasis group was significantly higher than that in the non-lymphatic metastasis group and non-tumor group (adjacent normal kidney group) (Figs. 3A and 3B).

Recombinant adenovirus ZD55-LASP-1 selectively inhibited LASP-1 expression in RCC cells
Next, we constructed ZD55-LASP-1 by inserting LASP-1 shRNA expression cassette into ZD55 adenovirus (Fig. 4A). Since E1A protein is essential for adenovirus replication, we performed Western blot analysis and found that the E1A protein level was significantly higher in 786-0 cells than in normal renal cells HK-2 after ZD55-LASP-1 infection (Fig. 4B). However, there was no difference in the E1A protein level between cells infected with ZD55-LASP-1 and ZD55-EGFP, showing that ZD55-LASP-1 did not interfere with viral replication.

In addition, Western blot analysis showed that ZD55-LASP-1 infection significantly decreased the LASP-1 protein level in 786-0 cells (Figs. 4C and 4D). These results demonstrated that ZD55-LASP-1 could effectively deplete LASP-1 in 786-0 cells.

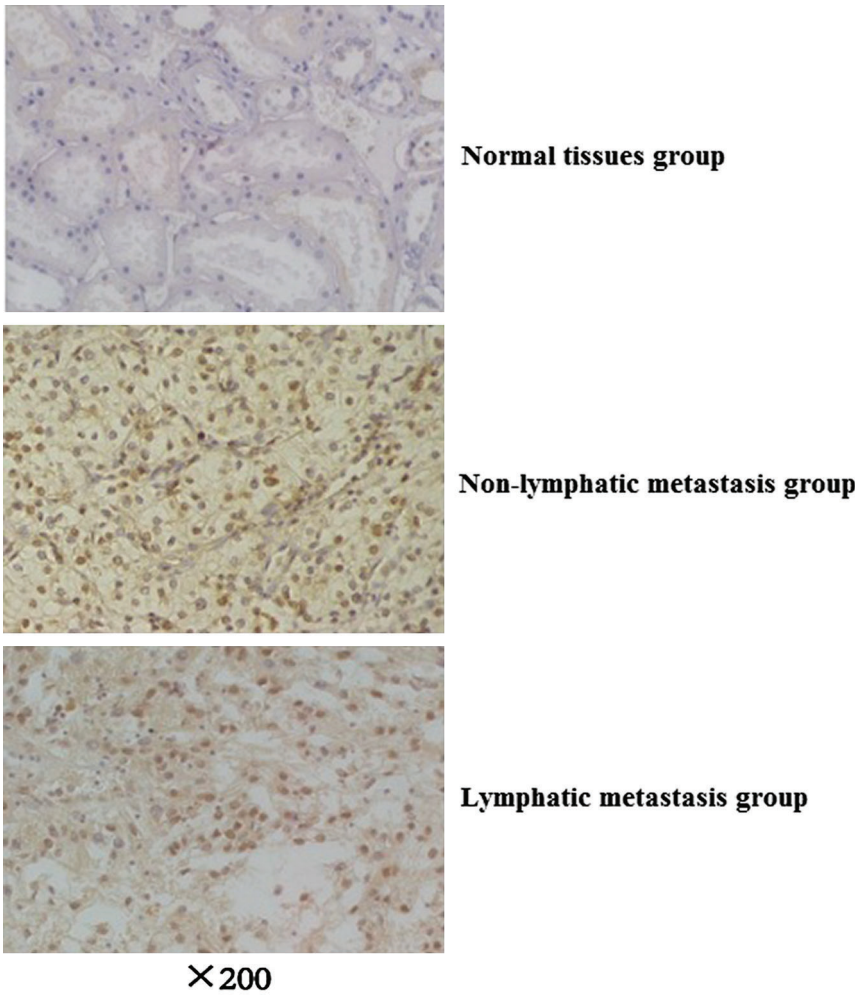


FIGURE 1. LASP-1 protein expression in normal renal and RCC tissues with or without lymph node metastasis. Representative immunohistochemical images (200×).

ZD55-LASP-1 inhibited the viability, migration, and invasion of RCC cells

CCK-8 assay showed that the cell viability of the ZD55-LASP-1 group was significantly lower than that of the ZD55-EGFP group and blank group (Fig. 5A). Transwell migration assay showed that the number of 786-0 cells that migrated the membrane in the ZD55-LASP-1 group was significantly less compared to the ZD55-EGFP group and blank group (Figs. 5B and 5C). Matrigel invasion assay demonstrated that the number of 786-0 cells that invaded the membrane in the ZD55-LASP-1 group was significantly less compared to the ZD55-EGFP group and blank group (Figs. 5D and 5E).

Furthermore, we performed AnnexinV-FITC/PI staining and found that the apoptosis rate of ZD55-LASP-1 treated group was significantly higher than that in ZD55-EGFP-treated group (19.134 ± 0.871 % vs. 8.932 ± 0.663 %, $P < 0.05$) and PBS group (8.724 ± 0.745 %, $p < 0.05$) (Figs. 5F and 5G).

ZD55-LASP-1 inhibited epithelial–mesenchymal transition (EMT) of RCC cells

Interestingly, LASP-1 was identified as a partner of vimentin in human hepatocellular carcinoma cells (Salvi *et al.*, 2015). To explore the mechanism of how LASP-1 regulated RCC cell invasion, we examined the expression of EMT-related proteins, including vimentin. We found that the E-cadherin protein level was significantly higher, while N-cadherin and vimentin protein levels were significantly lower in the ZD55-LASP-1 group, compared to the ZD55-EGFP group and blank group (Figs. 5H and 5I).

ZD55-LASP-1 exhibited antitumor efficacy in vivo

Next, we wanted to confirm the anti-tumor effects of ZD55-LASP-1 *in vivo*. Using nude mice subcutaneously injected with 786-0 cells as the model, we demonstrated that the anti-tumor activity of ZD55-LASP-1 was better compared to ZD55-EGFP and Ad-LASP-1 (Figs. 6A and 6C). The tumor volume of ZD55-LASP-1 group was 355.4 ± 47.4 mm^3, smaller than that of ZD55-EGFP group (541.2 ± 56.1 mm^3, $p < 0.05$), Ad-LASP-1 group (720.1 ± 74.5 mm^3, $p < 0.05$) and saline group (1051.1 ± 122.7 mm^3, $p < 0.05$). E1A expression was detected in ZD55-LASP-1 and ZD55-EGFP groups but not in the Ad-LASP-1 group (Fig. 6B), confirming viral replication in tumor tissues.

To verify that the anti-tumor effect was due to knockdown of LASP-1, LASP-1 expression in tumor tissues was analyzed by immunohistochemical staining. The results showed significantly reduced expression of LASP-1 in the ZD55-LASP-1 group compared to other groups (Figs. 6D and 6E).

Discussion

LASP-1 is overexpressed in many malignancies, but its expression in RCC remains unclear (Schreiber *et al.*, 1998;

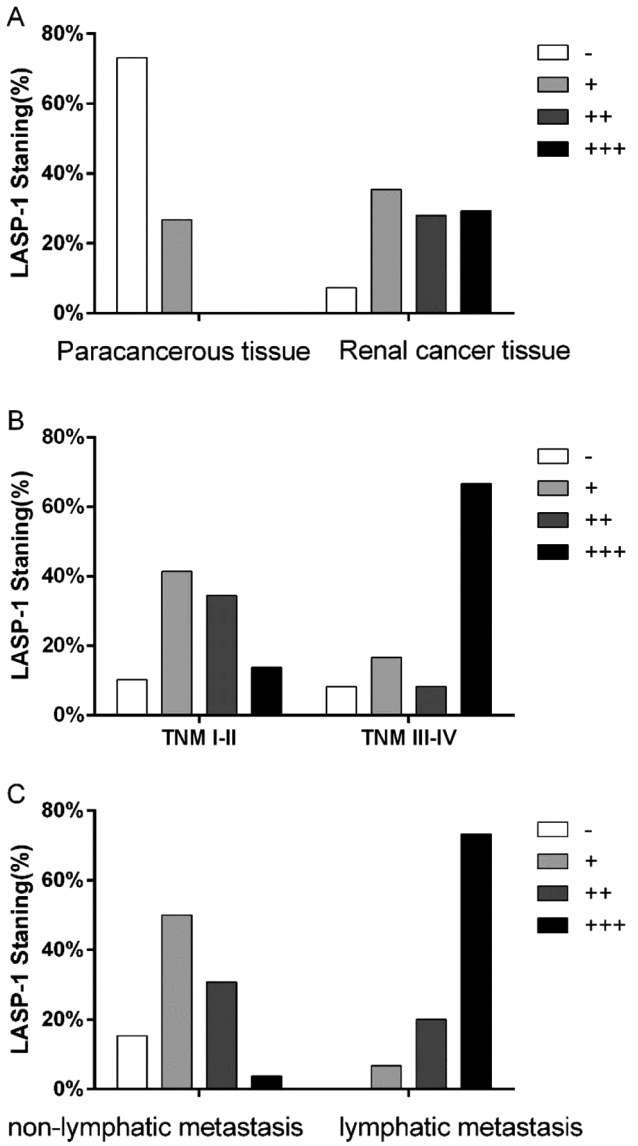


FIGURE 2. (A) LASP-1 protein levels in RCC and paracancerous tissues ($Z = -9.473$, $p < 0.001$). (B) Increased LASP-1 level was correlated with TNM stage ($r = 0.386$, $p < 0.001$). (C) Increased LASP-1 level was correlated with lymphatic metastasis ($r = 0.711$, $p < 0.001$).

TABLE 1

LASP-1 expression and clinicopathologic parameters of RCC patients

parameter	cases	LASP-1 expression (cases)				Statistics	*p*-value
		−	+	++	+++		
Tissue type							
RCC	82	6	29	23	24	$r = 0.742$	$p < 0.001$
Paracancerous tissue	82	60	22	0	0	$Z = -9.473$	$p < 0.001$
Tumor size (cm)							
<7	52	4	16	14	18		
≥7	30	4	12	8	6	$Z = -1.550$	$p = 0.121$
TNM stage							
I–II	58	6	24	20	8	$r = 0.386$	$p < 0.001$
III–IV	24	2	4	2	16	$Z = -3.473$	$p = 0.001$
Furhman grade							
I–II	40	4	24	12	8		

Table 1 (continued).

parameter	cases	LASP-1 expression (cases)				Statistics	*p*-value
		−	+	++	+++		
III	24	2	4	6	8		
IV	18	2	2	6	4	$\chi 2 = 5.019$	$p = 0.081$
lymph node metastasis							
+	30	0	2	6	22	$r = 0.711$	$p < 0.001$
−	52	8	26	16	2	$Z = -6.401$	$p < 0.001$

Note: $r > 0$ indicated positive correlation between the two groups.

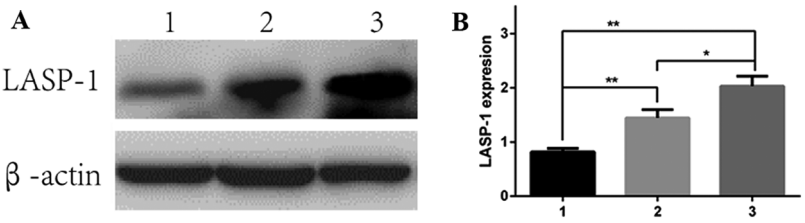
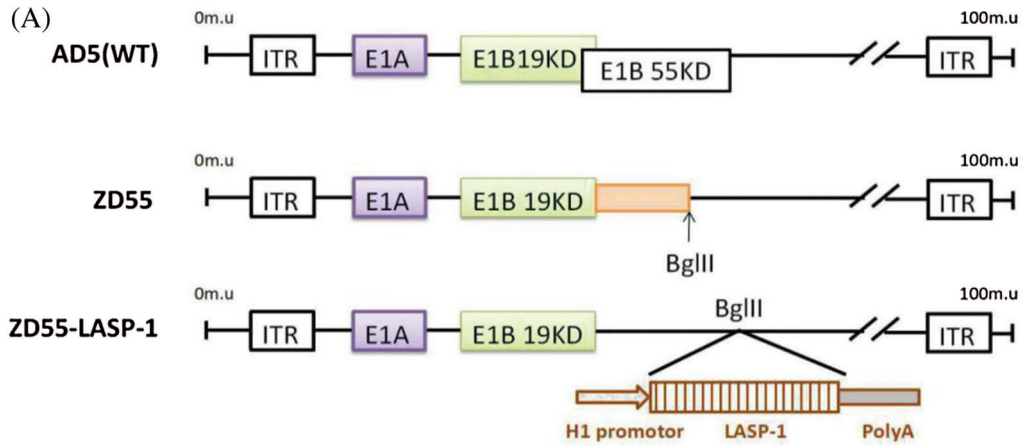


FIGURE 3. (A) The expression of LASP-1 in different tissues. 1. Adjacent normal renal tissue (non-tumor group). 2. RCC without lymph node metastasis (non-lymphatic metastasis group). 3. RCC with lymph node metastasis (lymphatic metastasis group). (B) Densitometry analysis of the LASP-1 level. *p < 0.05, **p < 0.01.



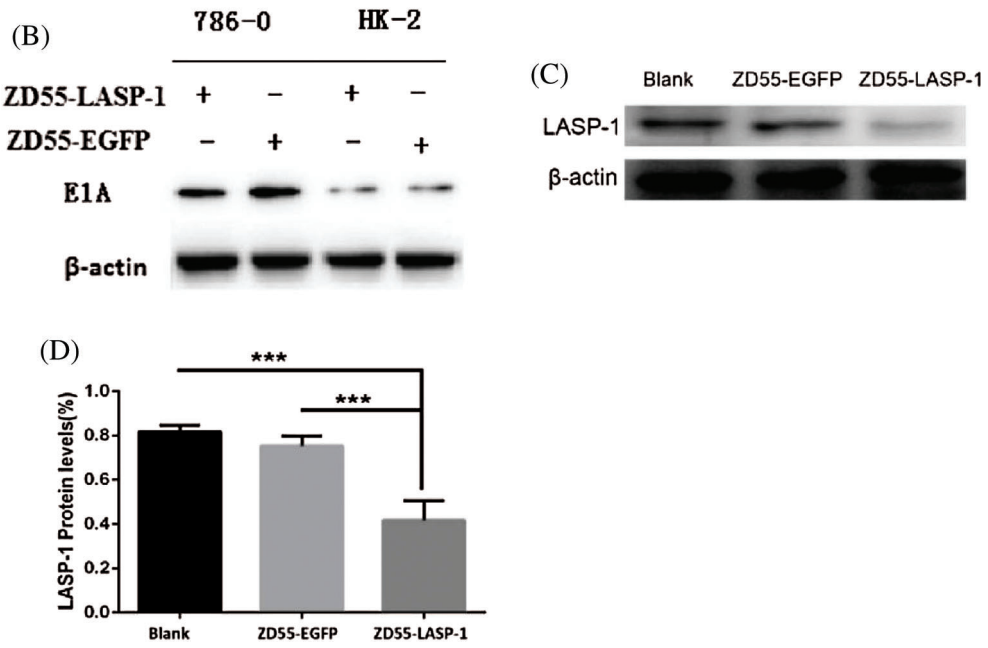


FIGURE 4. Construction of recombinant adenoviruses. (A) Schematic diagrams of adenoviruses ZD55 and ZD55-LASP-1. In ZD55-LASP-1, the E1B 55-kDa gene fragment was replaced by the LASP-1 shRNA cassette. (B) Western blot analysis of E1A expression in 786-0 and HK-2 cells infected by ZD55-LASP-1 and ZD55-EGFP and the statistical analysis of the expression of E1A. (C) Western blot analysis of LASP-1 expression in 786-0 and HK-2 cells infected by ZD55-LASP-1 and ZD55-EGFP. (D) LASP-1 protein level was significantly lower in the ZD55-LASP-1 group compared to the ZD55-EGFP group and blank group. ***p < 0.001.

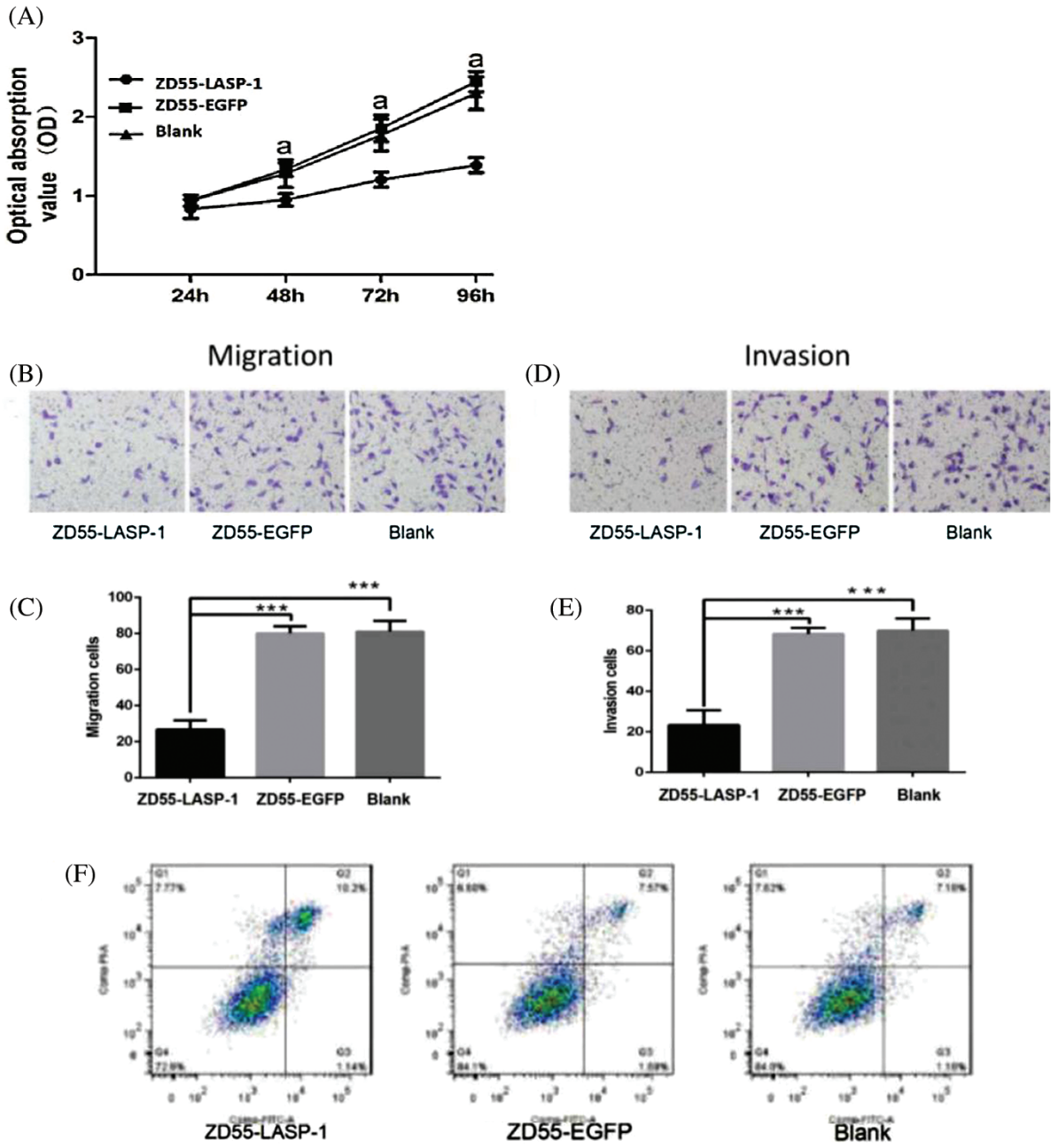


FIGURE 5. (continued)

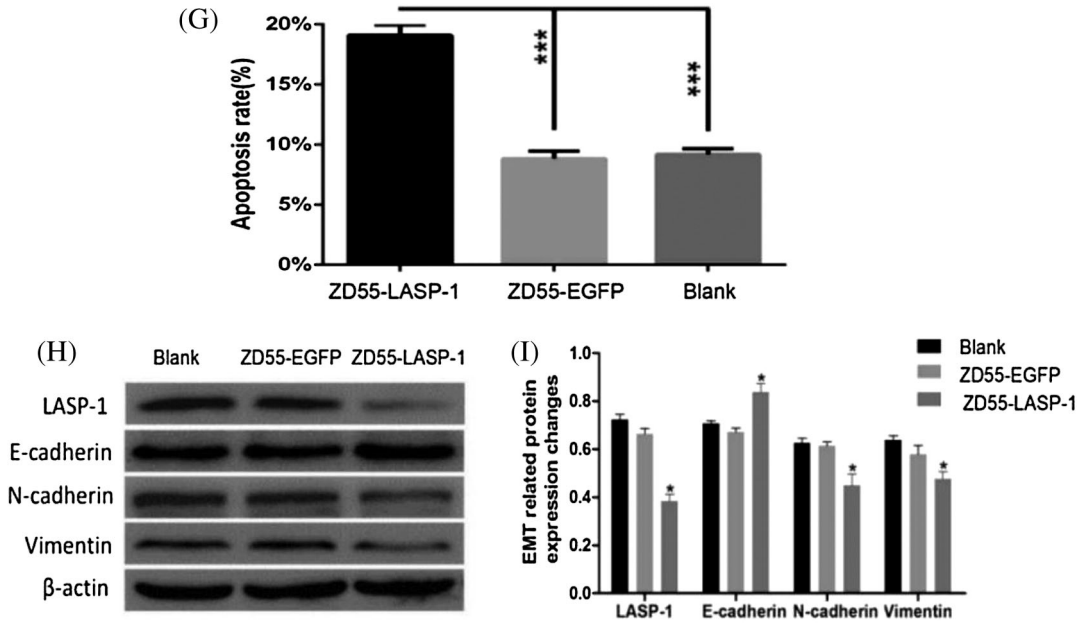


FIGURE 5. ZD55-LASP-1 inhibited malignant behaviors of RCC cells. (A) The proliferation rate of ZD55-LASP-1 group was significantly lower compared to ZD55-EGFP group and blank group (a, $p < 0.05$). (B, D) Invasion and migration of 786-0 cells after infection with viruses. Magnification: 200×. (C, E) The number of migrating and invading cells in each group. (F) Flow cytometry analysis of apoptosis of 786-0 cells. (G) Calculated apoptosis rate in each group. (H) Western blot analysis of the expression of EMT-related proteins in each group. (I) Densitometry analysis of protein levels. $^{*}p < 0.05$, $^{***}p < 0.001$.

Keicher *et al.*, 2004; Chew *et al.*, 2002; Butt *et al.*, 2003). Therefore, we speculated that LASP-1 may promote RCC. This study showed that LASP-1 was highly expressed in renal carcinoma tissues and cells. In addition, we found that LASP-1 expression was correlated positively with lymph node metastasis and TNM stage of RCC.

Accumulating evidence indicates that high expression of LASP-1 is essential to cancer progression and metastasis (Rachlin and Otey, 2006; Frietsch *et al.*, 2010; Yang and Tian, 2019). In particular, Yang *et al.* (2014) reported that LASP-1 was overexpressed in RCC tissues, and its expression level was correlated with overall survival and recurrence-free survival in RCC patients. Furthermore, knockdown of LASP-1 gene inhibited RCC cell migration *in vitro* (Yang *et al.*, 2014). However, whether LASP-1 is a potential therapeutic target for RCC *in vivo* remains to be confirmed. Therefore, in this study, we employed oncolytic adenovirus containing LASP-1 shRNA to test the combined effects of LASP-1 knockdown and the tumor-killing ability of oncolytic adenovirus. We constructed ZD55-LASP-1, which was an E1B 55-kDa deficient oncolytic adenovirus containing LASP-1 shRNA and showed that ZD55-LASP-1 exhibited synergistic anti-tumor efficacy based on oncolytic adenovirus and LASP-1 knockdown. To our knowledge, this is the first study on targeting LASP-1 for RCC therapy using *in vivo* animal model.

ZD55-LASP-1 mediated knockdown of LASP-1 in RCC cells led to decreased proliferation and increased apoptosis. These data suggested that LASP-1 could promote the development of RCC by increasing cell viability and decreasing cell apoptosis. One of the most important steps in tumor metastasis is the invasion of tumor cells and the acquisition of migration ability (Ren and Liang, 2019; Yang and Tian, 2019). We found that the migration and invasion ability of 786-0 cells was significantly lower in cells infected with ZD55-LASP-1, which suggested that high expression of LASP-1 might increase RCC cell migration and invasion. It is known that cancer cell invasion is regulated by EMT. In order to determine whether LASP-1 can affect cell invasion and migration through the EMT process, we performed Western blot analysis and found that ZD55-LASP-1 upregulated the expression of E-cadherin while downregulated the expression of N-cadherin and Vimentin. These results indicate that LASP-1 could promote the RCC invasion by enhancing the process of EMT.

In conclusion, LASP-1 was highly expressed in renal carcinoma cells and tissues, and the expression of LASP-1 was positively correlated with TNM stage and lymph node metastasis of RCC. Oncolytic adenovirus-mediated knockdown of LASP-1 significantly inhibited the proliferation, migration, and invasion of RCC cells and increased the apoptosis of RCC cells. Taken together, we conclude that LASP-1 plays an oncogenic role in RCC growth and metastasis, and it may become a potential target for RCC therapy.

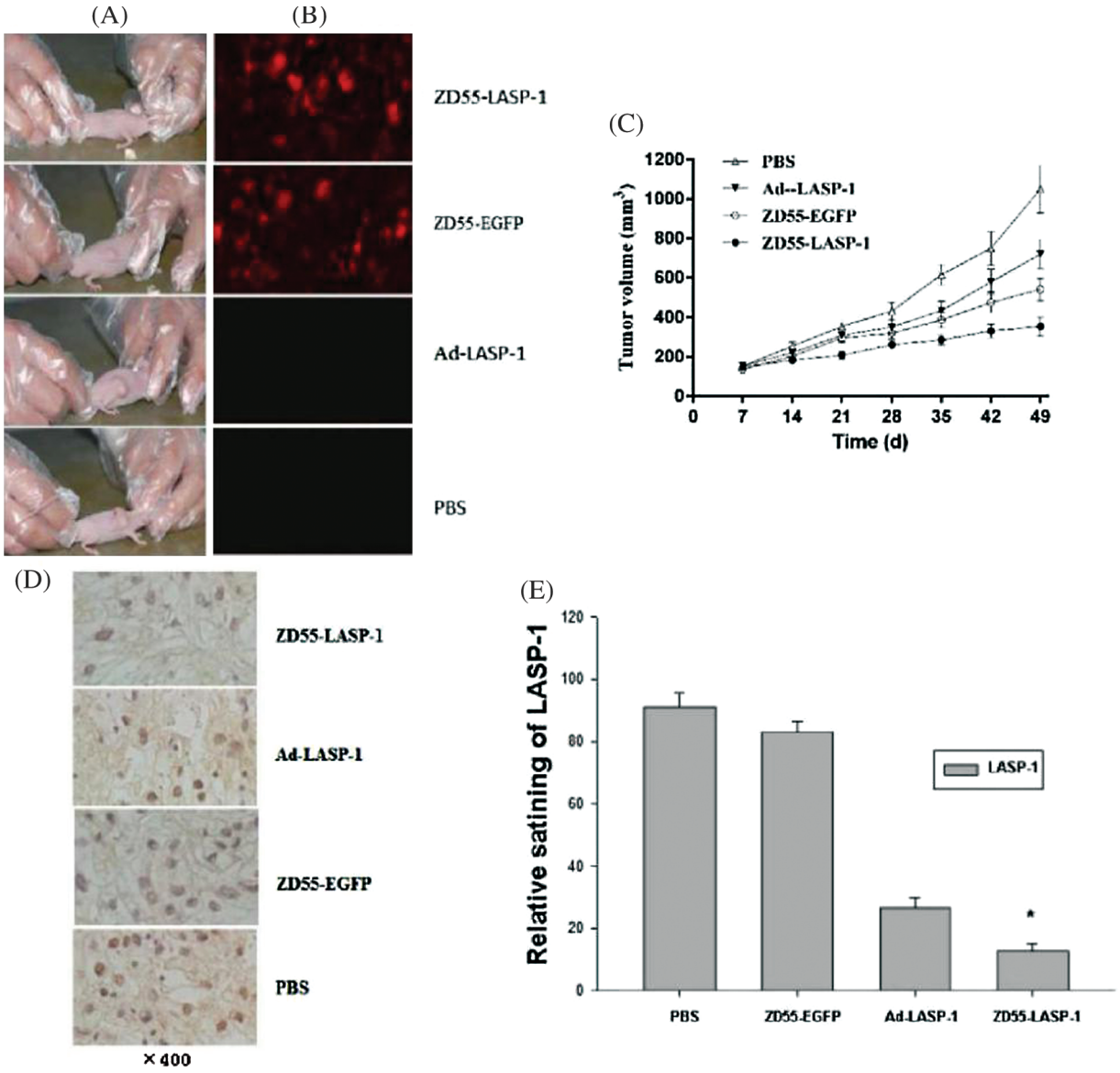


FIGURE 6. Anti-tumor efficacy of ZD55-LASP-1 *in vivo*. (A) Representative tumor xenografts from each group. (B) E1A expressions were detected by Laser Scanning Confocal Microscope. (C) Tumor growth curves of tumors following treatment as indicated (N = 5). *$p < 0.05$: ZD55-LASP-1 *vs.* other groups. (D) Immunohistochemical analysis of tumor sections. (E) Quantitative analysis of LASP-1 staining in xenografts from each group (N = 5). *$p < 0.01$: ZD55-LASP-1 *vs.* other groups.

Availability of Data and Materials: All data generated or analysed during this study are included in this article.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

Bex A, Gore M, Mulders P, Sternberg CN (2012). Recent advances in the treatment of advanced renal cell carcinoma: towards multidisciplinary personalized care. *BJU International* **110**: 1289–1300. DOI 10.1111/j.1464-410X.2012.11100.x.

Butt E, Gambaryan S, Göttfert N, Galler A, Marcus K, Meyer HE (2003). Actin binding of human LIM and SH3 protein is regulated by cGMP- and cAMP-dependent protein kinase phosphorylation on serine 146. *Journal of Biological Chemistry* **278**: 15601–15607. DOI 10.1074/jbc.M209009200.

Butt E, Raman D (2018). New frontiers for the cytoskeletal protein LASP1. *Frontiers in Oncology* **8**: 245. DOI 10.3389/fonc.2018.00391.

Chew CS, Chen X, Parente JA Jr, Tarrer S, Okamoto C, Qin HY (2002). Lasp-1 binds to non-muscle F-actin *in vitro* and is localized within multiple sites of dynamic actin assembly *in vivo*. *Journal of Cell Science* **115**: 4787–4799. DOI 10.1242/jcs.00174.

Frietsch JJ, Grunewald TG, Jasper S, Kammerer U, Herterich S, Kapp M, Honig A, Butt E (2010). Nuclear localisation of LASP-1 correlates with poor long-term survival in female breast cancer. *British Journal of Cancer* **102**: 1645–1653. DOI 10.1038/sj.bjc.6605685.

Grunewald TG, Kammerer U, Kapp M, Eck M, Dietl J, Butt E, Honig A (2007a). Nuclear localization and cytosolic overexpression of LASP-1 correlates with tumor size and nodal-positivity of human breast carcinoma. *BioMed Central Cancer* **7**: 198.

Grunewald TG, Kammerer U, Winkler C, Schindler D, Sickmann A, Honig A, Butt E (2007b). Overexpression of LASP-1 mediates migration and proliferation of human ovarian cancer cells

and influences zyxin localisation. *British Journal of Cancer* **96**: 296–305. DOI 10.1038/sj.bjc.6603545.

Kammerer-Jacquet SF, Thierry S, Rioux-Leclercq N (2018). Renal cell carcinoma: new histopathologic and evolutive renal entities. *Oncologie* **20**: 193–198. DOI 10.3166/onco-2019-0025.

Keicher C, Gambaryan S, Schulze E, Marcus K, Meyer HE, Butt E (2004). Phosphorylation of mouse LASP-1 on threonine 156 by cAMP- and cGMP-dependent protein kinase. *Biochemical and Biophysical Research Communications* **324**: 308–316. DOI 10.1016/j.bbrc.2004.08.235.

Leibovich BC, Lohse CM, Crispen PL, Boorjian SA, Thompson RH, Blute ML, Cheville JC (2010). Histological subtype is an independent predictor of outcome for patients with renal cell carcinoma. *Journal of Urology* **183**: 1309–1316. DOI 10.1016/j.juro.2009.12.035.

Rachlin AS, Otey CA (2006). Identification of palladin isoforms and characterization of an isoform-specific interaction between Lasp-1 and palladin. *Journal of Cell Science* **119**: 995–1004. DOI 10.1242/jcs.02825.

Ren J, Liang Q. (2019). HMGB1 promotes the proliferation and invasion of oral squamous cell carcinoma via activating epithelial-mesenchymal transformation. *Biocell* **43**: 199–206.

Salvi A, Bongarzone I, Ferrari L, Abeni E, Arici B, De Bortoli M, Scuri S, Bonini D, Grossi I, Benetti A, Baiocchi G, Portolani N, De Petro G (2015). Molecular characterization of LASP-1 expression reveals vimentin as its new partner in human hepatocellular carcinoma cells. *International Journal of Oncology* **46**: 1901–1912. DOI 10.3892/ijo.2015.2923.

Schreiber V, Moog-Lutz C, Régnier CH, Chenard MP, Boeuf H, Vonesch JL, Tomasetto C, Rio MC (1998). Lasp-1, a novel type of actin-binding protein accumulating in cell membrane extensions. *Molecular Medicine* **4**: 675–687. DOI 10.1007/BF03401929.

Simonaggio A, Rivallin P, Marret G, Oudard S, Vano YA (2018). Definfing the therapeutic strategy in metastatic renal cell carcinoma. *Oncologie* **20**: 211–219. DOI 10.3166/onco-2019-0026.

Yang C, Tian Y. (2019). SPAG9 promotes prostate cancer growth and metastasis. *Biocell* **43**: 207–214.

Yang F, Zhou X, Du S, Zhao Y, Ren W, Deng Q, Wang F, Yuan J (2014). LIM and SH3 domain protein 1 (LASP-1) overexpression was associated with aggressive phenotype and poor prognosis in clear cell renal cell cancer. *PLoS One* **9**: e100557. DOI 10.1371/journal.pone.0100557.

Zhao T, Ren H, Li J, Chen J, Zhang H, Xin W, Sun Y, Sun L, Yang Y, Sun J, Wang X, Gao S, Huang C, Zhang H, Yang S, Hao J (2015). LASP1 is a HIF1α target gene critical for metastasis of pancreatic cancer. *Cancer Research* **75**: 111–119. DOI 10.1158/0008-5472.CAN-14-2040.