LIM and SH3 Protein 1 Induces TGFβ-Mediated Epithelial–Mesenchymal Transition in Human Colorectal Cancer by Regulating S100A4 Expression

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Abstract

Purpose: The expression of LIM and SH3 protein 1 (LASP1) was upregulated in colorectal cancer cases, thereby contributing to the aggressive phenotypes of colorectal cancer cells. However, we still cannot decipher the underlying molecular mechanism associated with colorectal cancer metastasis.

Experimental Design: In this study, IHC was performed to investigate the expression of proteins in human colorectal cancer tissues. Western blot analysis was used to assess the LASP1-induced signal pathway. Two-dimensional difference gel electrophoresis was performed to screen LASP1-modulated proteins and uncover the molecular mechanism of LASP1. TGFβ was used to induce an epithelial–mesenchymal transition (EMT).

Results: LASP1 expression was correlated with the mesenchymal marker vimentin and was inversely correlated with epithelial markers, namely, E-cadherin and β-catenin, in clinical colorectal cancer samples. The gain- and loss-of-function assay showed that LASP1 induces EMT-like phenotypes in vitro and in vivo. S100A4, identified as a LASP1-modulated protein, was upregulated by LASP1. Moreover, it is frequently coexpressed with LASP1 in colorectal cancer. S100A4 was required for EMT, and an increased cell invasiveness of colorectal cancer cell is induced by LASP1. Furthermore, the stimulation of TGFβ resulted in an activated Smad pathway that increased the expression of LASP1 and S100A4. The depletion of LASP1 or S100A4 expression inhibited the TGFβ signaling pathway. Moreover, it significantly weakened the proinvasive effects of TGFβ on colorectal cancer cells.

Conclusion: These findings elucidate the central role of LASP1 in the TGFβ-mediated EMT process and suggest a potential target for the clinical intervention in patients with advanced colorectal cancer. Clin Cancer Res; 20(22); 5835–47. ©2014 AACR.

Introduction

Colorectal cancer is the third most common malignancy all over the world. Colorectal cancer is the leading cause of cancer death in this world. The incidence of colorectal cancer is increasing in China. Despite recent advances in the diagnosis and therapy of colorectal cancer, the general survival rate of patients with colorectal cancer has not improved. Metastasis is the main cause of mortalities and poor prognosis (1, 2). The molecular mechanisms underlying colorectal cancer metastasis are not quite clear till date.

Tumor metastasis is a complex process involving multiple pathogenic steps. Numerous molecules participate in tumor invasion and metastasis. However, we have not been yet been successful in deciphering the molecular changes associated with the acquisition of metastatic potential in colorectal cancer progression. Recent research studies have illustrated that the epithelial–mesenchymal transition (EMT) is involved in tumor progression, which is an important mechanism promoting tumor metastasis. EMT is a biologic process that permits a polarized epithelial cell to undergo multiple biochemical changes, thereby enabling it to assume a mesenchymal cell phenotype (3). Cancer cells undergoing EMT acquire aggressive properties and enter the surrounding stroma, thereby resulting in enhanced migratory capacity, invasiveness, and elevated resistance to apoptosis (4). Therefore, we must explore key molecules in EMT, which may be used to design new diagnostic strategies and specific targeted drugs for managing colorectal cancer metastasis.
Translational Relevance

Colorectal cancer is the third most common malignancy and the leading cause of cancer death in the world. Metastasis is the main cause of mortalities and poor prognosis. The expression of LIM and SH3 protein 1 (LASP1) was upregulated in colorectal cancer cases, especially aggressive colorectal cancer cases, thereby contributing to the central role in the aggressive phenotypes of colorectal cancer cells. Our present results reveal that LASP1 induces a TGFβ-mediated epithelial–mesenchymal transition in human colorectal cancer by regulating S100A4 expression. This is the first report that identifies the molecular mechanisms underlying LASP1 in colorectal cancer metastasis. Thus, our study provides new insights and strategies into the targeted therapeutic intervention for advanced colorectal cancer based on the functions of LASP1.

LIM and SH3 protein 1 (LASP1) was initially identified from metastatic axillary lymph nodes of patients with breast cancer. LASP1, a specific focal adhesion protein, is involved in several biologic and pathologic processes (5, 6). The upregulated expression of LASP1 has been found in several types of cancers (7–9). In a previous study, we illustrated the decreased expression of miR133a in colorectal cancer tissues and confirmed that miR133a inhibited LASP1 expression by directly binding with its 3′ untranslated region (3′ UTR) in colorectal cancer cells (10). Furthermore, we validated the overexpression of LASP1 in colorectal cancer tissue. We also found that the expression of this protein was closely correlated with lymph node metastasis, thereby improving the overall survival rates of patients with colorectal cancer. An overexpression of LASP1 created aggressive phenotypes of cancer cells, thereby promoting cancer growth and metastasis (11). These results indicated that LASP1 might be a promising molecule that could be used in developing treatments for patients with colorectal cancer. Presently, we do not have any documented evidence that elucidates the molecular mechanism of LASP1 in colorectal cancer metastasis.

In this study, we investigate the involvement of LASP1 in EMT of colorectal cancer by examining its relation with EMT-associated markers in human colorectal cancer tissue samples. We also determined its role on TGFβ-induced EMT and cell invasion in colorectal cancer cell lines while searching for mechanisms underlying its effect in colorectal cancer. We wanted to deepen our understanding of colorectal cancer metastasis and provide the experimental basis for targeted treatment of patients with advanced colorectal cancer.

Materials and Methods

Cell culture and treatment

Colorectal cancer cell lines HCT116, SW480, and SW620 were obtained from the ATCC and maintained as described previously (11). The cells were cultured in RPMI 1640 (Hyclone), which was supplemented with 10% FBS (Gibco-BRL; Invitrogen) at a humidity of 5% CO₂ at 37°C. For TGFβ treatment, the cells were stimulated using 10 ng/ml human recombinant TGFβ1 (Peprotech), which was diluted with serum-free medium containing BSA for time periods of 24 and 48 hours.

A plasmid containing LASP1 cDNA was kindly donated by Dr. Elke Butt (Institute of Clinical Biochemistry and Pathobiocchemistry, University of Wuerzburg, Germany). LASP1-specific siRNAs were synthesized as previously described (11). Exponential growth phase cells were plated in 6-well plates at a density of 0.5 × 10⁶ cells/mL, cultured for 24 hours, and transfected with 1 mg of siRNA or 4 μg cDNA in reduced serum medium (OPTI-MEM-I; Invitrogen) according to the manufacturer’s protocol. Fluorescein (FAM)-labeled negative control siRNA was used to visualize the transfection efficiency.

Tumor tissue sample

All cases of tumor tissue were provided by the Tumor Tissue Bank of Nanfang Hospital. Total 180 clinical samples were randomly selected to avoid sampling bias. Fresh-frozen tumor samples were selected from 14 of these patients, and Western blot analysis was performed on these samples. Formalin-fixed tumor tissues from the remaining 166 patients were used for the purpose of immunohistochemical analysis. In each case, a diagnosis of primary colorectal cancer was performed. In the period extending from 2001 to 2013, elective surgery was performed on patients with colorectal cancer in Nanfang Hospital. The Tumor Tissue Bank of Nanfang Hospital possesses a comprehensive set of clinicopathologic data, including age, gender, size of primary tumor, tumor differentiation, T classification, and N classification. Among them, complete follow-up data, ranging from 0 to 86 months, were available in 126 cases of patients with colorectal cancer for analyzing the relationship between LASP1 and clinicopathologic feathers. Other 40 cases of patients with colorectal cancer were used to investigate the relationship of LASP1 with EMT markers. The pathologic diagnosis was performed in the Department of Pathology of Nanfang Hospital of Southern Medical University. The study was approved by the Ethics Committee of Southern Medical University. All the aspects of this study were complied with the Declaration of Helsinki. In this study, the data would be analyzed anonymously. Therefore, this study did not require informed consent. This was specifically approved by the Ethics Committee of Southern Medical University.

Western blot analysis

The immunoblot analysis of cell lysates (20–60 μg) was carried out for assessing protein expression in RIPA buffer in the presence of mouse antibodies to LASP1 (1:2,000; Chemicon), S100A4 (1:500; Abcam), and β-actin, E-cadherin, β-catenin, vimentin, GAPDH (1:500; Santa Cruz Laboratories); and rabbit antibodies to p-Akt (Ser473), p-ERK1/2 (Thr202/Tyr204), p-ERK1/2 (Thr185/Tyr187), and p-p38 (Thr180/Tyr182). A plasmid containing LASP1 cDNA was kindly donated by Dr. Elke Butt (Institute of Clinical Biochemistry and Pathobiocchemistry, University of Wuerzburg, Germany). LASP1-specific siRNAs were synthesized as previously described (11). Exponential growth phase cells were plated in 6-well plates at a density of 0.5 × 10⁶ cells/mL, cultured for 24 hours, and transfected with 1 mg of siRNA or 4 μg cDNA in reduced serum medium (OPTI-MEM-I; Invitrogen) according to the manufacturer’s protocol. Fluorescein (FAM)-labeled negative control siRNA was used to visualize the transfection efficiency.
p-Akt, AKT, p-c-raf, p-MEK1/2, p44/42 MAPK (ERK1/2), p-p44/42 MAPK (ERK1/2), Smad2, p-Smad2 (1:1,000; CST).

**Immunohistochemistry**

As previously described, IHC was performed (12) to investigate the expression of proteins in human colorectal cancer tissues. The sections were incubated overnight using primary antibodies against LASP1, S100A4 (1:500), E-cadherin, β-catenin, vimentin (1:50) at 4°C. Mayer’s hematoxylin was used for the purpose of nuclear counter staining. In this study, these slides were reviewed by two or three blind-folded pathologists. To evaluate the expression levels of protein, the intensity of staining of cancer cells was scored as follows: 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellowish brown), and 3 (strong staining, brown). An intensity score of ≥2 was considered as high expression (or overexpression), whereas <2 in the intensity score was regarded as low expression. The discrepancies (≤5%) were resolved by simultaneous re-evaluation. The significance of correlation was determined using the Pearson χ² test.

**Results**

**LASP1 was related to tumor invasion and induced changes of colorectal cancer cells in cellular morphology**

To determine the expression of LASP1 in colorectal cancer tissues, IHC assay was first performed in 126 cases of all colorectal cancer tissues with complete follow-up data. Compared with the adjacent noncancerous mucosa, an overexpression of LASP1 was detected in colorectal cancer tissues. This was closely related with the metastasis of lymph nodes (Fig. 1A). Furthermore, we observed that LASP1 significantly upregulated the invasive margin that surrounded the microinvasive foci of colorectal cancer (Fig. 1B).

To establish a stably LASP1-overexpressing cell line SW480/LASP1, we transfected LASP1 cDNA into SW480 colorectal cancer cells. Interestingly, LASP-1 overexpression caused changes in cellular morphology under inverted microscopy and scanning electron microscopy (SEM). Compared with SW480/Mock cells, SW480/LASP1 cells displayed a long spindle-shaped form with thin, long pseudopods, which occasionally resembled finger-like pseudopods that extended from the cell bodies (Fig. 1C).

Furthermore, using experimental animal model, we observed primary tumor and liver metastatic lesions that were derived from subcutaneous and spleen injection, respectively. In the control group, tumors had a clear differentiation, whereas the invasive capacities of SW480 cells were obviously higher in SW480/LASP1 cells than SW480/Mock cells (Fig. 3B). Using IHC assay, we detected the protein expression of LASP1 and EMT markers in subcutaneous tumors, which were derived from SW480/LASP1 and

**LASP1 was correlated with expression of E-cadherin, β-catenin, and vimentin in colorectal cancer tissues**

Immunoblotting analysis was performed to detect the expression of LASP1, E-cadherin, β-catenin, and vimentin in colorectal cancer tissues. We found relatively high expression level of LASP1 in 71.4% (10/14) colorectal cancer tissues. Interestingly, the correlative expression of vimentin along with inversely correlative expression of E-cadherin and β-catenin was observed in 57.1% (8/14) colorectal cancer tissues (Fig. 2A).

In this study, the correlation between the expression of LASP1, E-cadherin, β-catenin, vimentin, and clinicopathologic parameters was analyzed to understand the clinical significance of these proteins in colorectal cancer tissues. Using IHC assay, we detected LASP1, E-cadherin, β-catenin, and vimentin expression in 40 other cases of all colorectal cancer tissues. There were no significant differences between LASP1 similar to E-cadherin, β-catenin, vimentin, and clinicopathologic feathers, except for E-cadherin and gender (P = 0.004), nucleus β-catenin and tumor site (P = 0.010), E-cadherin and tumor site (P = 0.006), vimentin and tumor differentiation (P = 0.002; Supplementary Table S1).

Furthermore, we analyzed the correlation of LASP1 expression with E-cadherin, β-catenin, and vimentin expression in colorectal cancer tissues, respectively. As shown in Fig. 2B and Supplementary Table S2, LASP1 was closely correlated with vimentin (r = 0.431, P = 0.006). But, it was inversely correlated with E-cadherin (r = −0.337, P = 0.033) and membrane β-catenin (r = −0.328, P = 0.039). However, there was no significant correlation between LASP1 expression and nucleus β-catenin (r = 0.006, P = 0.971; Fig. 2C).

**LASP1 enhanced cell invasiveness through induction of EMT-like changes**

To confirm whether LASP1 is required for colorectal cancer cell EMT and invasiveness, we performed gain- and loss-of-function assay using gene transfection and RNAi. After transfection of LASP1 cDNA, the cancer cells assumed EMT-like changes, which are indicated by the decreased expression of epithelial markers (E-cadherin and β-catenin) and the increased expression of mesenchymal marker (vimentin) in SW480 and HCT116 cells. In contrast, we found the RNAi-mediated gene silence of LASP1, which was inhibited by the EMT process in SW620 cells (Fig. 3A and C).

To investigate the role of LASP1 in the EMT process in vivo, we stably transfected SW480 cells with LASP1 cDNA for establishing LASP1-overexpressing colorectal cancer cells, which were named as SW480/LASP1. Transwell assays showed that the invasive capacities of SW480 cells were obviously higher in SW480/LASP1 cells than SW480/Mock cells (Fig. 3B). Using IHC assay, we detected the protein expression of LASP1 and EMT markers in subcutaneous tumors, which were derived from SW480/LASP1 and
control cells. With an increase of LASP1 expression, the primary tumors of the SW480/LASP1 group developed EMT-like changes that were comparable with the tumors that originated from SW480/Mock cells.

In previous studies, we found that miR133a directly suppressed LASP1 expression by binding to the 3' UTR of LASP1 (10). Furthermore, we confirmed whether knockdown of LASP1 inhibited EMT of colorectal cancer cells. IHC assay showed that the tumors in the SW480/miR133a group restored the expression of epithelial markers and inhibited the expression of mesenchymal marker. Meanwhile, along with the increase of LASP1 expression, the number of positive Ki-67 tumor cells significantly increased in tumors from SW480/miR133a to SW480/LASP1 groups (Fig. 3D).

**LASP1 regulated pivotal biologic processes and activated multiple signaling transduction pathways**

To explore the molecular mechanism underlying LASP1-mediated EMT, we performed two-dimensional difference
gel electrophoresis (2-D DIGE). This was done to analyze the effect of overexpression and silence the LASP-1 gene on protein expression profiles of SW480 and SW620 cells, respectively. Among the differentially expressed proteins, the expression level of 60 protein spots was consistently changed in SW480 and SW620 cells. Using Gene Ontology analysis, it was found that the candidate proteins integrated with several key biologic processes, such as morphogenesis, development, signal transduction, cell proliferation, and cell cycle. This explained the LASP1-mediated biologic behaviors (Supplementary Fig. S1).

This proteomic study revealed that LASP1 is involved in signaling transduction pathways. So, we carried out Western blot analysis for elucidating the phosphorylation status of proteins that are involved in EMT signaling. As shown in Fig. 4A, LASP1 significantly induced phosphorylation of c-raf, MEK, p44/42 MAPK (ERK1/2), Smad2, AKT at Ser473, and Thr308 in SW480 cells, thereby indicating that it can function as a key mediator of EMT via MAPK, PI3K/AKT, and Smad signaling.

To further elucidate the relationship between LASP1 and TGFβ signaling in colorectal cancer, we analyzed the effect of recombinant TGFβ on SW480, HCT116, and canine kidney derived epithelial cell line (MDCK) cells. MDCK is usually used in EMT study and undergoes typical morphologic changes of EMT, which is induced

Figure 2. LASP1 was correlated with expression of E-cadherin, β-catenin, and vimentin in colorectal cancer tissues. A, immunoblotting analysis is performed to detect the expression of LASP1, E-cadherin, β-catenin, and vimentin in colorectal cancer tissues. The samples with correlative expression of LASP1 and EMT markers are indicated (hollow arrow). B, the LASP1 expression is associated with vimentin, but it is inversely associated with E-cadherin and membrane β-catenin in 40 colorectal cancer specimens. We have shown the visualizations of two representative cases. Bottom plots are corresponding high magnification of top plots. C, the percentages of samples showing low or high LASP1 expression in 40 colorectal cancer specimens that were relative to the levels of vimentin, E-cadherin, or membrane β-catenin.
by TGFβ (13, 14). These results indicated that TGFβ stimulation was induced through a classical change of EMT markers, leading to a significant increase of LASP1 expression. The cells also illustrated a dynamic activation of Smad2 phosphorylation, but the expression levels of Smad2 were not affected by TGFβ treatment (Fig. 4B).

After being treated with TGFβ for 48 hours, the cells appeared spindle-shaped, and a fibroblastic-like phenotype appeared instead of the cobblestone-like phenotype (Fig. 4C). Coupled with the morphologic changes of EMT, we also observed time-dependent increases in the invasive ability of tumor cells (Fig. 4D).
S100A4 expression was upregulated by LASP1 and frequently coexpressed with LASP1 in colorectal cancer

Proteomic study revealed that one of the candidates of LASP1-modulated proteins was identified as S100A4, which was positively correlated with LASP1 (Fig. 5A). To accompany with upregulated LASP1, the enhanced intensity of S100A4 was found in SW480/LASP1 cells (labeled with Cy3) compared with that of the control cells (labeled with Cy5). Meanwhile, to accompany with decreased LASP1, the lower intensity of S100A4 was observed in SW480/siLASP1 cells (labeled with Cy5) compared with control cells (labeled with Cy3). The introduction of LASP1 cDNA resulted in the elevated expression of both protein and mRNA levels of S100A4 in SW480 and HCT116 cells. The further results showed that LASP1 did not affect protein stability and ubiquitin-mediated degradation of S100A4 (Supplementary Fig. S2). In contrast, the gene silencing of LASP1 obviously decreased S100A4 mRNA and protein expression in SW620 cells (Fig. 5B–D). With an increase of LASP1 expression, the primary tumors of the SW480/LASP1 group showed markedly higher S100A4 expression compared with the tumors that originated from control cells, especially that from the SW480/miR133 group (Fig. 5E).

Western blot technique was used to detect LASP1 and S100A4 expression in 14 cases of fresh colorectal cancer tissues. These results suggested that S100A4 expression was frequently higher in colorectal cancer samples with LASP1 overexpression (Supplementary Fig. S3A). To further investigate the expression and clinical significance of S100A4, IHC assay was performed to analyze the correlation of S100A4 expression with clinicopathologic parameters and LASP1 expression in colorectal cancer tissues. No significant difference was reported between S100A4 expression and clinicopathologic features, except for gender of patients with colorectal cancer (Supplementary Table S3). However, S100A4 expression was obviously correlated with LASP1 expression (Supplementary Fig. S3B; r = 0.553, P = 0.000). Meanwhile, a close correlation of S100A4 overexpression with low E-cadherin or negative membrane β-catenin expression was found, but not for nuclear β-catenin and vimentin (Supplementary Fig. S3C and Supplementary Table S4).

LASP1 and S100A4 were required by TGFβ-mediated EMT

To explore the role of S100A4 in LASP1-mediated EMT and the invasiveness of colorectal cancer cells, we simultaneously coinfected LASP1 cDNA and S100A4 siRNA into SW480 and HCT116 cells. We also observed the effects of S100A4 abolishment on LASP1-transfected cells. These results indicated that S100A4 siRNA neutralized the influence of LASP1 on cell phenotype. This resulted in the rescued expression of epithelial markers and weakened the expression of mesenchymal markers (Supplementary Fig. S4A). The wound-healing and Transwell assays illustrated that the knockdown of S100A4 counteracted with the LASP1-induced enhancement of cell invasiveness and migration (Supplementary Fig. S4B and S4C).

We investigated the role of LASP1 combined with S100A4 in the EMT process, which is involved in the TGFβ/Smad pathway. We also simultaneously transfected LASP1 or S100A4 siRNA and introduced TGFβ into SW480 and HCT116 cells. Similar with the LASP1 expression mentioned earlier, TGFβ-induced enhanced S100A4 expression of tumor cells was reported in a time-dependent manner (Fig. 6A). The transfection of LASP1 or S100A4 siRNA can partially eliminate EMT-like changes. Moreover, it can inactivate an EMT-associated signaling pathway that is induced by TGFβ in SW480 and HCT116 cells. Western blot analysis showed that LASP1 or S100A4 siRNA can obviously increase the expression of mesenchymal markers and suppress the phosphorylation of MEK, ERK, AKT, and Smad2. At the same time, it downregulated the expression of epithelial markers by TGFβ stimulation (Fig. 6B). The knockdown of LASP1 or S100A4 can also significantly neutralize the promoting effect of TGFβ on cell invasiveness in SW480 and HCT116 cells (Fig. 6C and D).

Discussion

Recent researchers have identified LASP1 as a tumor metastasis–associated protein in various types of cancer, such as breast (15), ovarian (9), bladder (16), hepatocellular cancer (17, 18), and medulloblastoma (19). In our previous studies, LASP1 was identified as a protein that was associated with the development and progression of colorectal cancer by proteomic analysis (20). An overexpression of LASP1 was found in metastatic colorectal cancer tissues, and its expression was closely correlated with the overall survival of patients with colorectal cancer. The gain of function and loss of function in vitro and in vivo also illustrated that LASP1 promoted tumor metastasis and induced aggressive phenotype of colorectal cancer cells, thereby indicating its key role in colorectal cancer progression (11). The results suggested that LASP1 is a promising target in clinical therapy of patients with advanced colorectal cancer. Unfortunately, the molecular mechanisms underlying LASP1 in colorectal cancer metastasis are still unclear.

EMT played a critical role in cancer progression and metastasis. Cancer cells undergoing EMT acquired invasive properties. Numerous studies have illustrated that EMT is a common molecular mechanism in colorectal cancer metastasis, and many proteins contribute to this process (21, 22). Taking this into consideration, we hypothesized that LASP1 is involved in EMT-mediated colorectal cancer metastasis. In this study, LASP1 expression was closely related with EMT markers in clinical colorectal cancer samples. In vitro and in vivo studies have shown that the introduction of LASP1 induced EMT-like phenotype changes. It also enhanced the capability of colorectal cancer cell invasiveness. Our results suggested...
that LASP1 probably participates in EMT-associated colorectal cancer progression. Thus, it provides a useful cue in uncovering the mechanism underlying colorectal cancer metastasis.

We used a high-throughput proteomic strategy to comprehensively explain LASP1-induced biologic processes. While comparing protein profiling of colorectal cancer cells treated with LASP1, the LASP1-modulated proteins...
were involved in several important biologic processes that played essential molecular functions. Among them, S100A4, a member of the S100 family, was positively regulated by LASP1 in colorectal cancer cells. S100A4 was overexpressed in several kinds of tumor, including gastric (23), hepatocellular (24), colorectal (25), lung (26), and breast carcinoma (27). This was recognized as colorectal cancer metastasis–associated protein and represented as a highly significant prognostic marker in colorectal cancer (28–31). In recent times, several research studies have focused on a crucial role of S100A4 in EMT (32, 33). The expression of S100A4 can be determined by paracrine factors similarly involved in EMT and cancer progression, including TGFβ signaling via Smad proteins and FGF-2 (34, 35). On the transcriptional level, the S100A4 gene is controlled by several regulatory elements and transcriptional factors, including β-catenin, a mediator of EMT, motility, and metastasis (36).

Despite the central role of LASP1 in colorectal cancer metastasis, the downstream effects of LASP1 continue to be unclear. In this study, S100A4 was identified as a LASP1-modulated protein. In clinical samples of colorectal cancer, there existed a significant correlation between LASP1 and S100A4 expression. In colorectal cancer cells, an introduction of LASP1 remarkably enhanced the expression of S100A4 mRNA and protein. On the other hand, S100A4 is required for TGFβ-mediated EMT. A, Western blot of S100A4 in indicated cells in response to the treatment with 10 ng/mL TGFβ for 0, 24, and 48 hours. B, the Western blot of LASP1, S100A4, vimentin, E-cadherin, p-MEK1/2, p-44/42 MAPK (ERK1/2), p-p44/42 MAPK (ERK1/2), p-Akt (Ser473), AKT, Smad2, and p-Smad2 in indicated cells that were coinfected with 10 ng/mL TGFβ and LASP1 or S100A4 siRNA. C and D, the representative figures and data of Transwell assay for SW480 (C) and HCT116 (D) cells coinfected with LASP1 vector and S100A4 siRNA. Each bar represents the mean ± SD. The results were reproduced in three independent experiments. E, a hypothetical model illustrating that LASP1 contributed to TGFβ-induced EMT by regulating S100A4.
hand, siRNA-mediated LASP1 obviously suppressed the expression of S100A4 mRNA and protein. Nuclear localization of LASP1 was found in breast cancer (15) and hepatocellular cancer (18). LASP1 contains LIM domain, which may potentially interact with transcriptional factors. In addition, our previous proteomic analysis demonstrated that LASP1 modulated protein profiling of colorectal cancer cells in a manner similar to "reprogramming" (11). The further results also showed that LASP1 did not affect protein stability and ubiquitin-mediated degradation of S100A4. The above-mentioned evidences support that LASP1 regulated S100A4 expression at transcriptional level, but not posttranscriptional level, protein stability, or degradation. Moreover, S100A4 was required for increasing the cell invasiveness in colorectal cancer cells that was induced by LASP1. This is because in colorectal cancer cells coinfected with LASP1 cDNA and S100A4 siRNA, the effects of LASP1 on cell invasion and migration were significantly reduced. We propose that S100A4 is a demonstrably downstream effector of LASP1 through which LASP1 exerts its proinvasive effects on colorectal cancer cells.

In this study, LASP1 can activate the MAPK, PI3K/AKT, and Smad signaling pathways by regulating the protein phosphorylation level, which were recognized as pivotal link of cancer progression. TGFβ-induced EMT via Smads and the complementary non-Smad pathway, such as MAPK (37–39) and PI3K/AKT (40, 41). In terms of MAPK, ERK activation is one of the Smad-independent events that are required for TGFβ-mediated EMT (42, 43). According to recent research studies, it was found that the abnormal activation of ERK played an important role in diverting the TGFβ response toward EMT in epithelial cells of kidneys. Previous studies have illustrated that ERK is rapidly activated by TGFβ in culture models of EMT. Furthermore, a specific inhibitor of MEK (upstream of ERK) blocks key morphologic features of EMT (44, 45). Raf activation confers protection against TGFβ-induced apoptosis by enhancing the proinvasive effects of TGFβ (46). Similarly, our data indicate that LASP1-induced activation of MAPK occurs via phosphorylation of c-Raf, MEK, and ERK.

Both LASP1 and S100A4 were closely related with the TGFβ/Smads pathway, a classical EMT mode. TGFβ-induced EMT-like phenotype changes and promoted colorectal cancer cell invasiveness and migration. We also observed a simultaneous increase in the expression of LASP1 and S100A4 that could be associated in a time-dependent manner. Moreover, LASP1 and S100A4 were required for increasing the cell invasiveness of colorectal cancer cells by TGFβ, because in colorectal cancer cells transfected with LASP1 and S100A4 siRNA, the effects of TGFβ on the cell invasion were reduced significantly. We propose that in colorectal cancer, LASP1 and S100A4 is a significant downstream effector of the TGFβ signaling pathway through which TGFβ exerts its proinvasive effects on colorectal cancer cells.

In this study, we found that the contribution of EMT to tumor metastasis is mediated mainly through an increased expression of LASP1 in colorectal cancer cells. In our previous study, miR1/133a suppressed the tumor growth and metastasis in colorectal cancer by directly targeting LASP1 3' UTR (10). DNA methylation inhibits the expression of miR1/133a, which could be indirectly upregulated with LASP1 expression (47). Moreover, the stimulation of TGFβ significantly induces the expression of LASP1 in a time-dependent manner. Thus, LASP1 overexpression was frequently found in colorectal cancer tissues, especially in metastatic colorectal cancer tissues. In this study, LASP1 can contribute to TGFβ-mediated EMT via the Smad-dependent and Smad-independent pathways. In the Smad-dependent pathway, LASP1 activates Smad2 by regulating S100A4 expression. Activated Smad2 formed complexes with common-partner Smad4 and translocated them into the nucleus (48). The complexes interacted with various transcription factors and transcriptional coactivators or corepressors and regulated the transcription of target genes (49, 50). In addition, TGFβ activated various types of non-Smad signaling in cancer cells. Among them, PI3K/AKT and MAPK played important roles in TGFβ-induced EMT (Fig. 6E).

In conclusion, LASP1 played a critical role in the TGFβ-mediated EMT process, which results in colorectal cancer metastasis. Thus, LASP1 is a central molecular regulator during colorectal cancer progression. Moreover, LASP1 may be used to design new diagnostic strategies and specific targeted drugs for managing the patients with advanced colorectal cancer.
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References


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