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Plastin 1 drives metastasis of colorectal cancer through the IQGAP1/Rac1/ERK pathway

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Abstract

Tumor metastasis is the dominant cause of death in colorectal cancer (CRC) patients, and it often involves dysregulation of various cytoskeletal proteins. Plastin 1 (PLS1) is an actin-bundling protein that has been implicated in the structure of intestinal epithelial microvilli; however, its role in CRC metastasis has not yet been determined. In this study, we demonstrated that PLS1 is highly expressed in 33.3% (45/135) of CRC patients and is correlated with lymph node metastasis and poor survival. In in vitro and in vivo experiments, PLS1 induced the migration and invasion of CRC cells and the metastases to the liver and lung in mice. Moreover, the expressions of key factors for CRC metastases, matrix metalloproteinase (MMP) 9 and 2, were enhanced by PLS1, which was dependent on phosphorylating ERK1/2 activated by IQGAP1/Rac1 signaling. The connection between these signals and PLS1 was further confirmed in CRC tissues of patients and the metastatic nodules from a mouse model. These findings suggest that PLS1 promotes CRC metastasis through the IQGAP1/Rac1/ERK pathway. Targeting PLS1 may provide a potential approach to inhibit the metastasis of CRC cells.

KEYWORDS

colorectal cancer, ERK1/2, IQGAP1, metastasis, PLS1

Tongtong Zhang, Zheng Wang and Yanjun Liu contributed equally to this work.

1 | INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies, and the third leading cause of cancer-related mortalities in the world.[1] Although advances have been made in the treatment of CRC over the past few decades, CRC is still a highly lethal disease. Death of cancer patients is attributed predominantly to metastasis. The mechanisms governing metastasis are very complicated; liver-limited metastasis of CRC alone involves over 750 genes.[2] Targeting metastasis-driving genes is an approach to treating cancer patients at late stage of disease.[3]

Plastins (also known as fimbrin) are actin-bundling proteins that contain three members, PLS1 (I-plastin), PLS2 (L-plastin) and PLS3 (T-plastin). They are essential for cytoskeleton stabilization, cell-cell adhesion, cell movement and migration, and regulate the stability of membrane proteins to influence cell behavior.[4] In recent 20 years, clinical studies have shown that PLS2 and PLS3 are associated with CRC metastasis and poor prognosis.[5-8]Using comparative genomic hybridization, we found that PLS1 is amplified in CRC[9]; however, its role in cancers has never been explored.

Meanwhile, the molecular gears of plastins adjusting cancer metastasis have seldom been investigated. Mimori et al reported that PLS3 induced epithelial-mesenchymal transition, a hallmark of cancer metastasis,[10] and is highly expressed in circulating CRC cells.[5,6] However, the signal transduction that mediates this effect is unknown. In breast cancer cells, PLS2 is dispensable to cell migration but is phosphorylated by the ERK/MAPK pathway.[11] The current known data appear far from elucidating the functions of plastins in cancers and their underlying mechanisms.

In this study, we found that PLS1 behaved like the other plastin members, promoting metastasis of CRC, and was associated with poor outcomes. In fact, PLS1 drove cell migration and metastasis of CRC cells by upregulating matrix metalloproteinases (MMP) 9 and 2, well-known metastatic regulators,[12] which was dependent on phosphorylating ERK1/2, activated by IQGAP1/Rac1 signaling. The relationship of these molecules was further confirmed in CRC tissues and the metastatic nodules of a mouse model.

2 | MATERIALS AND METHODS

2.1 | Patients and tissue specimens

We obtained 135 fresh tissue samples of CRC patients with matched distal colorectal mucosa from the Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (CAMS and PUMC) from 2009 to 2012. None of the patients had received any anticancer treatment before the operation. After the operation, the tumors were histologically classified and staged according to the tumor node metastasis (TNM) system, and the histological grade of tumors was defined according to the tumor differentiation. All patients signed informed consent forms provided by CAMS and PUMC for sample collection, research and analysis. Our study was approved by the Ethics Committee/Institutional Review Board of the Cancer Institute/Hospital, CAMS and PUMC (approval no. NCC2013RE-025).

2.2 | Immunohistochemistry

We performed immunohistochemistry (IHC) as described in our previous studies.[13] Briefly, 4-μm-thick sections were subjected to deparaffinization, antigen retrieval and blockage of non–specific binding, with the blockage performed by incubation with 10% normal goat serum for 15 minutes. The sections were incubated with primary antibodies for PLS1 (1:100, Proteintech) and IQGAP1 (1:200, Abcam) at 4°C overnight, and then with a biotinylated secondary antibody. Subsequently, slides were stained with 3,3-diaminobenzidine tetrahydrochloride. Each separate tissue core was scored on the basis of the staining intensity and the percentage of positive staining areas of cells.[14] The staining intensity was scored as 0 (negative), 1 (weak), 2 (medium) and 3 (strong). The percentage of positive staining areas of cells was defined using a scale of 0 to 4, where 0 represented <10%, 1 was 10%-25%, 2 was 26%-50%, 3 was 51%-75% and 4 was ≥76%. The final staining score was defined as the staining intensity times the percentage of positive staining areas of cells, with a low expression group (≤6) and a high expression group (>7).

2.3 | Cell lines and culture conditions

The human CRC SW620, SW1116, HCT-15, HCT-116, RKO, SW480, LOVO and SW48 cell lines were purchased from the Typical Culture Preservation Commission Cell Bank, Chinese Academy of Sciences. All the cells were maintained at 37°C in 5% CO_2 in RPMI 1640 medium (Invitrogen) supplemented with 10% FBS.

2.4 | Transfection of lentivirus in colorectal cancer cells

Lentiviral constructs expressing PLS1 and sh-PLS1 were purchased from GenePharma (Shanghai, China). Colorectal cancer cells were grown in 24-well plates and transfected with 50 μL of constructed lentivirus vectors. Then, 24 hours post-transfection, cells were transferred to 25 cm^2 culture bottles and resistant clones were selected by culturing the cells for 1 week in 2 μg/mL puromycin medium.

2.5 | Western blot analysis

Total protein was extracted from cells using radio immunoprecipitation assay lysis buffer. Equal amounts of protein were separated by SDS-PAGE in a 10% gel and transferred to a nitrocellulose membrane. Proteins were detected using an enhanced chemiluminescence

system according to the manufacturer's instructions. Membranes were incubated overnight with the following primary antibodies: anti–ERK1/2 and anti–p-ERK1/2 (Cell Signalling Technology), anti–IQGAP1 (BD Biosciences), anti–PLS1 and anti–Rac1 (Proteintech).

2.6 | Boyden chamber assay

Cell migration assays were performed using 6.5 mm Transwells with 8.0 μm pore polycarbonate membrane inserts (Corning). Cells were starved for 24 hours in a serum-free medium and then seeded in the same medium in the upper compartment of the chamber with 2×10^4 cells in 0.1 mL medium. The lower chamber was filled with 600 mL RPMI 1640 medium supplemented with FBS. After incubation for 24 hours, cells that migrated to the underside of the membrane were fixed with methanol and stained with crystal violet. The cell invasion assay was performed similarly, except that the cells were seeded onto a Matrigel (BD Biosciences)-coated filter.

2.7 | Quantitative reverse RT-PCR

Total RNA was reverse-transcribed into cDNA with random primers using a Transcriptor First Strand cDNA Synthesis Kit (Roche), following the manufacturer's instructions. MMP expression was measured by quantitative RT-PCR using the FastStart Essential DNA Green Master (Roche) on the Roche Light Cycler 480 (Roche). RNA expression was normalized to human GAPDH. Primers are listed in Table S1.

2.8 | Immunofluorescence microscopy

Immunofluorescence staining was performed as described previously.[15]

In brief, colon cancer cells were fixed in 4% v/v paraformaldehyde for 10 minutes and permeated in 0.1% Triton X-100 for 10 minutes. Nonspecific binding was blocked by incubating the cells with 5% normal donkey serum in PBS for 10 minutes. Subsequently, cells were incubated with antibodies against PLS1 and IQGAP1, probed with goat anti–rabbit and goat anti–mouse secondary antibody (Jackson ImmunoResearch), and counterstained with DAPI (Merck). Cells were analyzed using a Zeiss LSM880 confocal microscope.

2.9 | Xenograft assays in nude mice

In tail vein injection-based in vivo metastasis assays, 6-week-old female nude mice (Vital River) were injected with 2×10^6 stably transfected SW620 cells per animal in their tail vein. For the liver metastatic assay, a single intrasplenic injection of 2×10^6 colorectal cells was administered. The mice were killed 30 days after injection and were examined for metastatic nodules in the lung and liver. The tissues were fixed in Bouin's solution, embedded in paraffin, cut into 5-mm sections, and stained with H&E.

2.10 | Statistical analysis

All statistical analyses were conducted using SPSS software (SPSS). The association between protein expression and various clinicopathological parameters was evaluated with the χ^2-test. Student's *t* test was used to analyze the results expressed as mean ± SD. For the survival analysis, the Kaplan-Meier (K-M) method was used to plot survival curves, and the differences between the curves were tested using log-rank tests. The significance of various prognostic factors was studied using a Cox regression model. Nomograms were generated according to the Cox regression coefficients of selected variables, and the predictive accuracy of every nomogram was evaluated with calibration plots. Nomograms and calibration plots were generated using R in R Studio (Version 1.1.447). Differences were considered statistically significant when P was <0.05.

3 | RESULTS

3.1 | Plastin 1 is overexpressed in colorectal cancer patients and correlates with metastases and poor prognosis

We investigated the expression of PLS1 in 135 cases of CRC tissues and paired adjacent non–tumor colorectal tissues using an IHC assay. The PLS1 protein mainly accumulated in the margin of cancer cells. PLS1 was expressed in only 5.2% (7/135) of the normal colorectal epithelium samples, but a significantly higher expression level of PLS1 was observed in 33.3% (45/135) of the colorectal cancer samples ($P < 0.01$, Figure 1A,B and Table 1). We also examined the protein levels of PLS1 in three tumor cases and normal tissue by western blotting. The PLS1 protein was clearly elevated in cancer tissues compared to adjacent non–tumor tissues (Figure 1C).

Meanwhile, the expression of PLS1 showed positive relationships with the clinicopathological characteristics of the patients. As described in Table 1, PLS1 was significantly correlated with lymph node metastasis ($P < 0.05$). Kaplan-Meier analysis of the data showed that patients with high PLS1 expression had significantly shorter disease-free survival and overall survival than patients with low expression ($P < 0.01$, Figure 1D,E). Furthermore, a multivariate Cox regression analysis confirmed that PLS1 expression could be an independent predictor of shorter disease-free survival and overall survival (Tables 2 and 3). Next, we established a nomogram using clinical features, including Grade, T, N, Stage, and PLS1 expression (Figure 1F,G). The bias-corrected lines in the calibration plot were close to the ideal curve (the 45° line), indicating good agreement between prediction and observation (Figure 1H,I). These results indicated that the high expression of PLS1 might be related to migration and invasion of CRC cells.

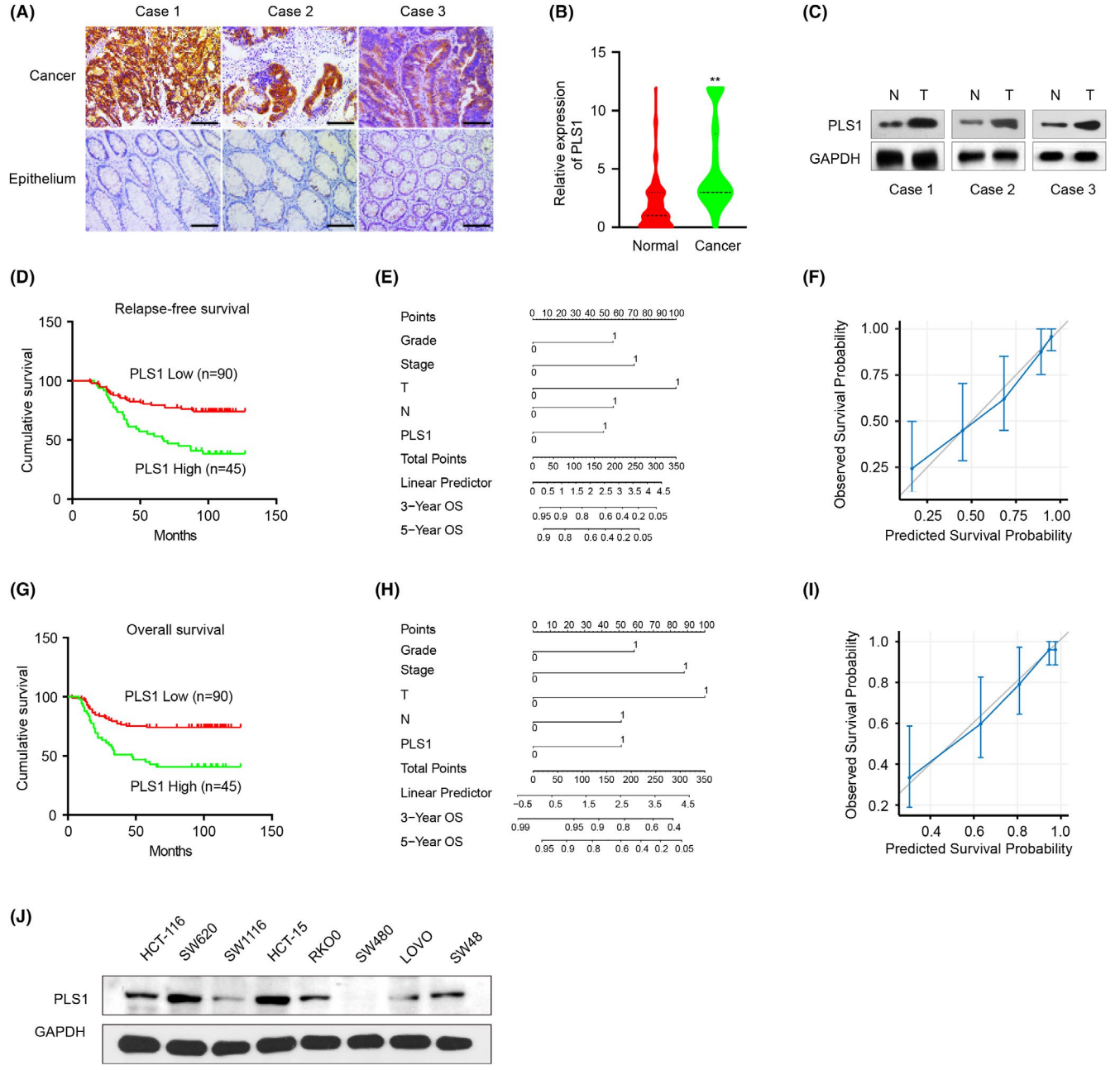

FIGURE 1 High expression of plastin 1 (PLS1) is associated with poor clinical outcome in colorectal cancer patients. A, Representative pictures of PLS1 expression in colorectal cancer and matching epithelium tissues by immunohistochemical analysis. B, PLS1 relative expression was analyzed in colorectal cancer tissues and the paired normal mucosal tissues using immunohistochemistry scoring standards. C, PLS1 expression was analyzed in colorectal cancer tissues and the paired normal mucosal tissues using western blot assay. The Kaplan-Meier analysis is used to examine relapse-free survival (RFS) times (D) and overall survival (OS) times (E) of colorectal cancer patients. Nomograms predicting relapse-free survival (RFS) (F) and overall survival (OS) (G) of colorectal cancer patients were created based on expression of PLS1 and several other clinical prognosis factors. Predicted and actual relapse-free survival (RFS) (H) and overall survival (OS) (I) probabilities for every nomogram were compared using calibration plot in which circles denote nomogram-predicted probabilities and error bars represent the 95% confidence interval of these estimates. For an ideal nomogram, the predictive survival rates should fall on the 45° diagonal line. J, PLS1 expression in colorectal cancer cell lines by western blot analysis. N, normal tissue; T, cancer tissue. Scale bars, 100 μm; error bars, means ± SD (n = 3 independent experiments). *$P < 0.05$; **$P < 0.01$; not significant

3.2 | Plastin 1 is required for colorectal cancer cell invasion and migration in vitro

To confirm whether PLS1 induces metastasis, we performed in vitro experiments using eight CRC cell lines. Western bolt assay showed that the PLS1 had high expression in SW620 and HCT-15 cells (Figure 1J) but low expression in SW480 and SW1116 cells. To explore the role of the PLS1 protein in CRC cell migration and invasion, we constructed cell lines in which PLS1 expression was either artificially elevated or suppressed, by using lentivirus

vectors or short hairpin RNA (sh-PLS1). Western blot analysis showed that PLS1 was successfully knocked down in SW620 and HCT-15 cells, and it was overexpressed in SW480 and SW1116 cells (Figure S1). To examine the effect of PLS1 on the motility of CRC cells, we carried out migration and invasion assays with SW620-sh-PLS1 and HCT-15-sh-PLS1 cells for 24 hours. Results showed that PLS1-silenced cells had lower capacity for migration and invasion than control cells (Figure 2A,B). In addition, high levels of PLS1 facilitated cell migration and invasion in SW480 and SW1116 cells compared with control cells (Figure 2C,D).

TABLE 1 PLS1 expression in colorectal cancer

Clinical or molecular feature	Total N	PLS1		
		Low	High	*P*
	135	90	45	
Age (mean ± SD)				
<62	67	43	24	0.433
≥62	68	47	21	
Gender				
Male	77	51	26	0.944
Female	58	39	19	
Grade				
Low	113	77	36	0.398
High	22	13	9	
Stage				
I + II	75	57	18	0.012
III	60	33	27	
Tumour volume				
≤15 cm^3	71	52	19	0.103
>15 cm^3	64	38	26	
Tumour location				
Colon	33	15	18	0.002
Rectum	102	75	27	
T				
T3	124	85	39	0.195
T4	11	5	6	
N				
N0	75	59	18	0.012
N1 + N2	60	33	27	

3.3 | Plastin 1 promotes colorectal cancer cell metastasis in vivo

Because the liver and lung are the most affected organs in CRC metastasis,[15,17] we observed the effects of PLS1 on both liver and lung metastases of CRC cells xenotransplanted into NOD/SCID mice. SW620 cells transfected with sh-PLS1 or negative control shRNA lentivirus were introduced via the tail vein into nude mice in each group. Six weeks after injection, animals were killed and examined for the presence of lung metastases.

In the control sequence RNAi group, the mice developed lung nodules that were visible to the naked eye. The average number of lung metastases in the mice injected with SW620 cells transfected with sh-PLS1 was significantly lower than that in the mice injected with cells transfected with control shRNA (Figure 2E).

For studying in vivo invasion following injection of SW480 cells, we used a mouse model of liver metastasis in which cancer cells were injected into the spleen of nude mice. The development of liver metastases was evaluated grossly and histologically when the mice were killed after 7 weeks. Compared with control SW480 cells, SW480 cells overexpressing PSL1 showed increased metastasis to the liver in nude mice (Figure 2F).

3.4 | Plastin 1 regulates matrix metalloproteinases through the ERK pathway

Matrix metalloproteinase 9 is a critical player in invasion and metastasis of CRC.[18-21] Hence, we sought to investigate whether MMP are the target of PLS1 signaling that regulates cell invasion and migration. Our data showed that knockdown of PLS1 in SW620 and HCT-15 cells inhibited the expression of MMP2 and MMP9. Conversely,

TABLE 2 Univariate and multivariate analyses for relapse-free survival (Cox proportional hazards regression model)

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Gender (male vs female)	0.811	0.450-1.460	0.484	–	–	–
Age (≤62 y vs >62 y)	1.066	0.601-1.888	0.828	–	–	–
Location (colon vs rectum)	1.257	0.663-2.384	0.483	–	–	–
Volume (≤15 cm^3 vs >15 cm^3)	1.446	0.813-2.571	0.209	–	–	–
Stage (phage)	7.514	3.620-15.595	<0.001	–	–	–
Grade (low vs high)	2.449	1.289-4.651	0.006	2.680	1.377-5.215	0.004
T (T2 + T3 vs T4)	3.425	1.593-7.364	0.002	5.675	2.477-13.006	<0.001
N (N0 vs N1 + N2)	7.514	3.620-15.595	<0.001	7.674	3.595-16.382	<0.001
PLS1 (high vs low expression)	3.183	1.788-5.666	<0.001	2.153	1.197-3.871	0.010

TABLE 3 Univariate and multivariate analysis for overall survival (Cox proportional hazards regression model)

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Gender (male vs female)	0.859	0.473-1.560	.618	–	–	–
Age (≤62 y vs >62 y)	1.008	0.562-1.810	.978	–	–	–
Location (colon vs rectum)	1.303	0.683-2.483	.422	–	–	–
Volume (≤15 cm^3 vs >15 cm^3)	1.622	0.897-2.931	.109	–	–	–
Stage (phage)	8.414	3.904-18.131	<.001	–	–	–
Grade (low vs high)	2.498	1.308-4.770	.006	2.787	1.426-5.447	0.003
T (T2 + T3 vs T4)	3.706	1.716-8.002	.001	6.855	2.890-16.256	<0.001
N (N0 vs N1 + N2)	8.414	3.904-18.131	<.001	9.242	4.111-20.779	<0.001
PLS1 (high vs low expression)	3.504	1.935-6.346	<.001	2.248	1.227-4.119	0.009

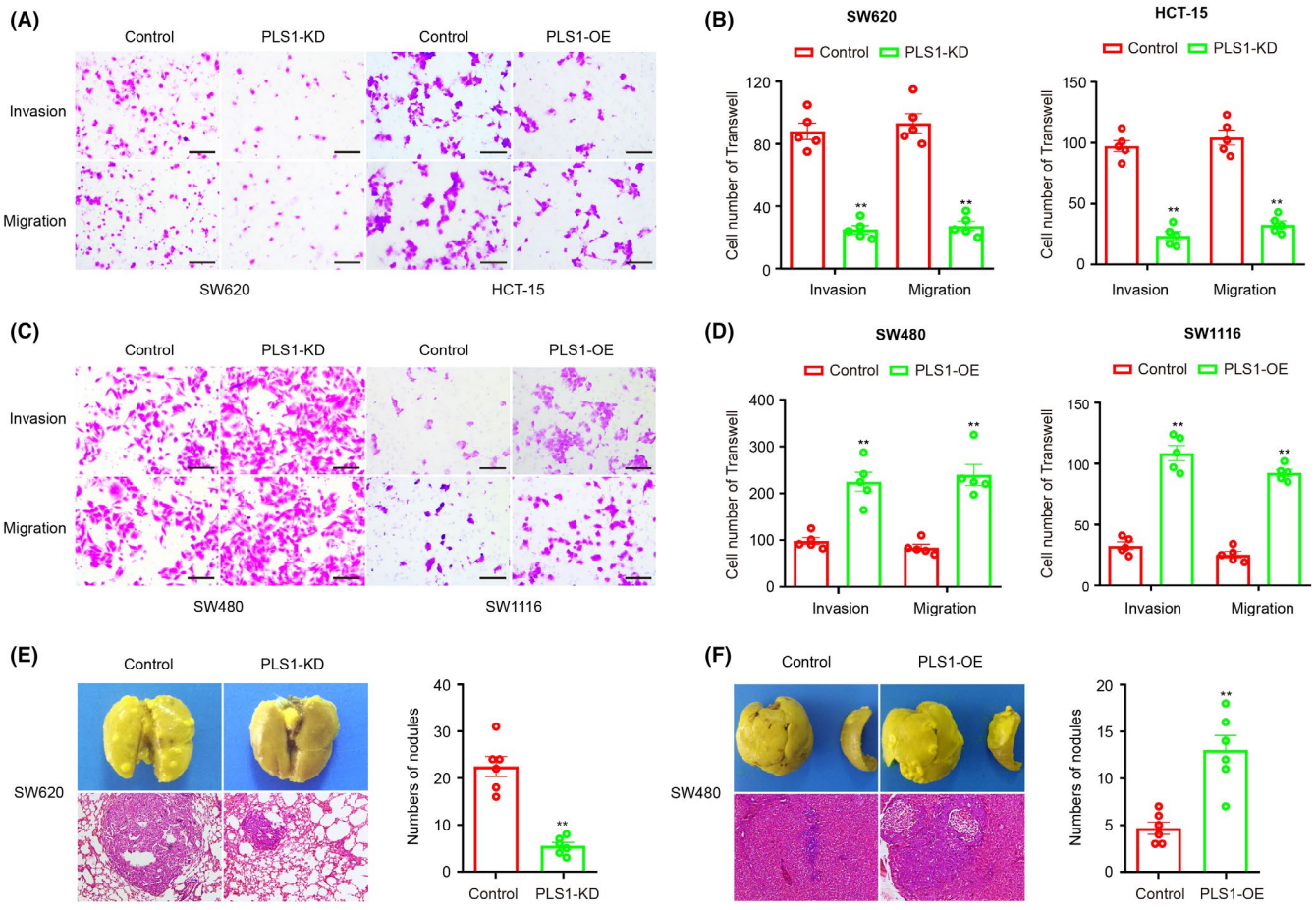

FIGURE 2 Plastin 1 (PLS1) promotes colorectal cancer cell metastasis in vitro and in vivo. A, Migration and invasion ability of PLS1 overexpression SW620 and HCT-15 cells was detected following PLS1 knockdown by the transwell assay. B, Number of transwell cells was quantified to detect migration and invasion of SW620 and HCT-15 cells. C, Migration and invasion ability of PLS1 downregulation SW480 and SW1116 cells was detected following PLS1 overexpression by the transwell assay. D, Number of transwell cells was quantified to detect migration and invasion of SW480 and SW1116 cells. E, Mice were injected with SW620 cells via the tail vein. After 30 d, the mice were killed. The lungs were then photographed after Bouin's fixation (top), and sections of the lungs were stained with H&E (bottom). Number of lung metastases present in the mice 10 wk post–injection was examined by eye. F, Mice were injected with SW480 cells via the spleen and were killed 10 wk later. The livers were then photographed after Bouin's fixation (top) and sections of the liver were stained with H&E (bottom). The number of liver metastases present in the mice 10 wk post–injection was examined by eye. Scale bars, 100 μm; error bars, means ± SD (n = 3 independent experiments). **P* < 0.05; ***P* < 0.01; not significant

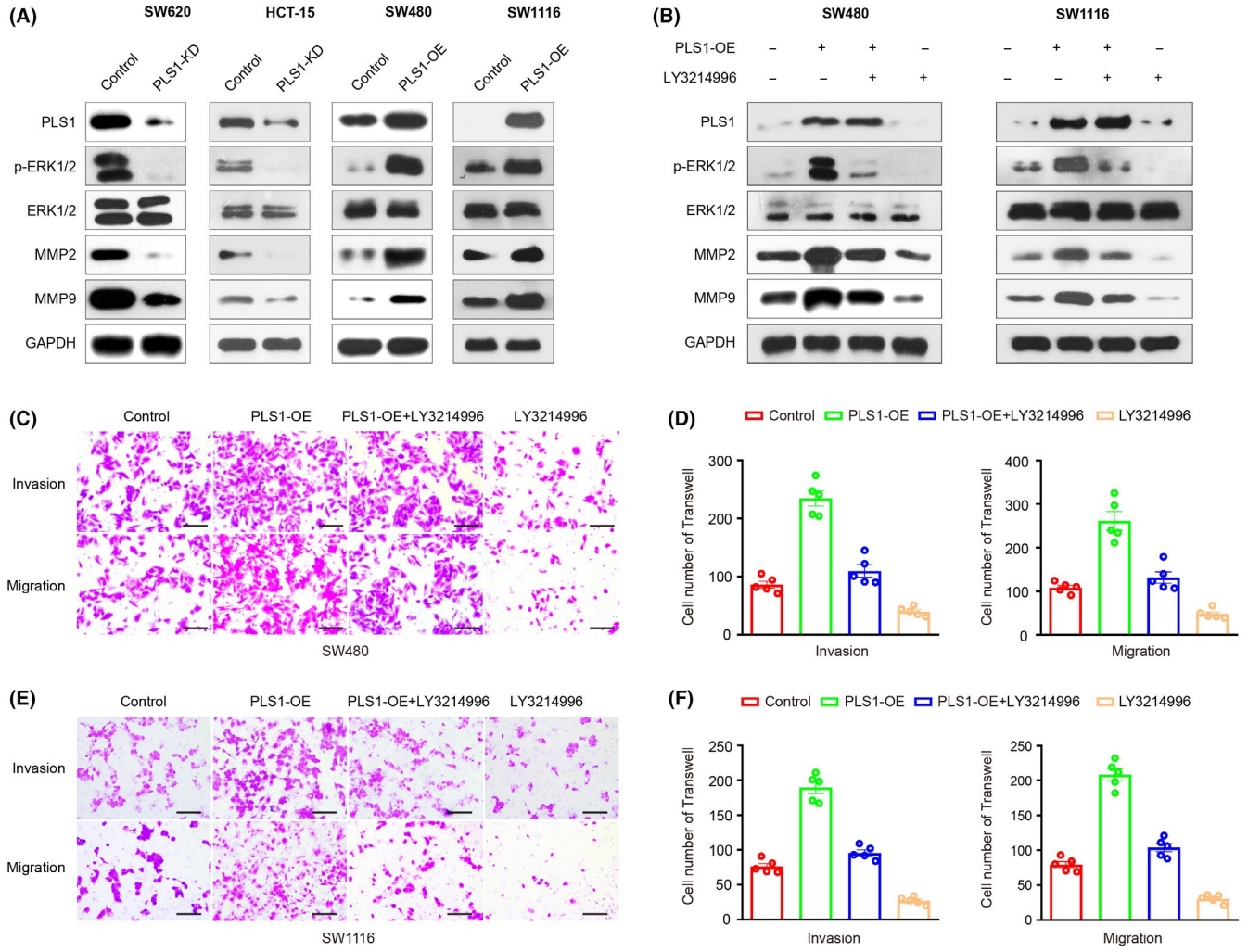

FIGURE 3 Plastin 1 (PLS1) promotes colorectal cancer cell invasion and migration depends on ERK activity. A, Downregulation of PLS1 expression inhibits the expression of p-ERK1/2 in SW620 and HCT-15 cells, whereas upregulation of PLS1 elevates the expression of p-ERK1/2 in SW480 and SW1116 cells, as shown by western blot analysis. B, ERK1/2 inhibitor LY3214996 partially reversed the p-ERK1/2 and downstream protein upregulation in SW480 and SW1116 cells with PLS1 overexpression, as shown by western blot analysis. C, ERK1/2 inhibitor LY3214996 partially reversed the invasion and migration capacity of SW480 cells with PLS1 overexpression. D, The number of transwell cells was quantified to detect migration and invasion of SW480 cells. Scale bars, 100 μm. E, ERK1/2 inhibitor LY3214996 partially reversed the invasion and migration capacity of SW1116 cells with PLS1 overexpression. D, The number of transwell cells was quantified to detect migration and invasion of SW1116 cells. Scale bars, 100μm. Error bars, means ± SD (n = 3 independent experiments)

upregulation of PLS1 in SW480 and SW1116 cells elevated MMP2 and MMP9 levels (Figure 3A and Figure S2).

Studies have shown that the ERK pathway of upstream MMP is a key signaling pathway regulating cell invasion and migration, which can be significantly activated in CRC.[22,23] To determine whether the ERK pathway is involved in the effect of PLS1, we used PLS1-knockdown SW620 and HCT-15 cells and PLS1-overexpressing SW480 and SW1116 cells to evaluate the protein levels of ERK signaling pathway-related genes by western blotting. The results showed that knockdown of PLS1 in SW620 and HCT-15 cells inhibited the expression of phosphorylated ERK1/2 (p-ERK1/2). Conversely, upregulation of PLS1 in SW480 and SW1116 cells activated the ERK pathway (Figure 3A).

To further confirm whether the activation of the ERK pathway is necessary for the function of PLS1, we used a selective ERK1/2 inhibitor LY3214996 to abolish the signal transduction of PLS1 in CRC cells. We found that the p-ERK1/2 protein levels were increased after PLS1 overexpression. However, upon co–treatment with LY3214996, there was a decrease in the phospho-ERK1/2 level. Moreover, the induction of the invasion markers MMP2 and MMP9 was reversed in response to LY3214996 treatment (Figure 3B and Figure S3). Subsequently, the PLS1-induced cell migratory and invasive abilities were also impeded (Figure 3C-F).

3.5 | Plastin 1 promotes ERK activity depending on the IQGAP1-Rac1 pathway

ERK activity is regulated by the binding of the cytoskeleton-related protein IQGAP1.[21] We have previously reported that IQGAP1 is

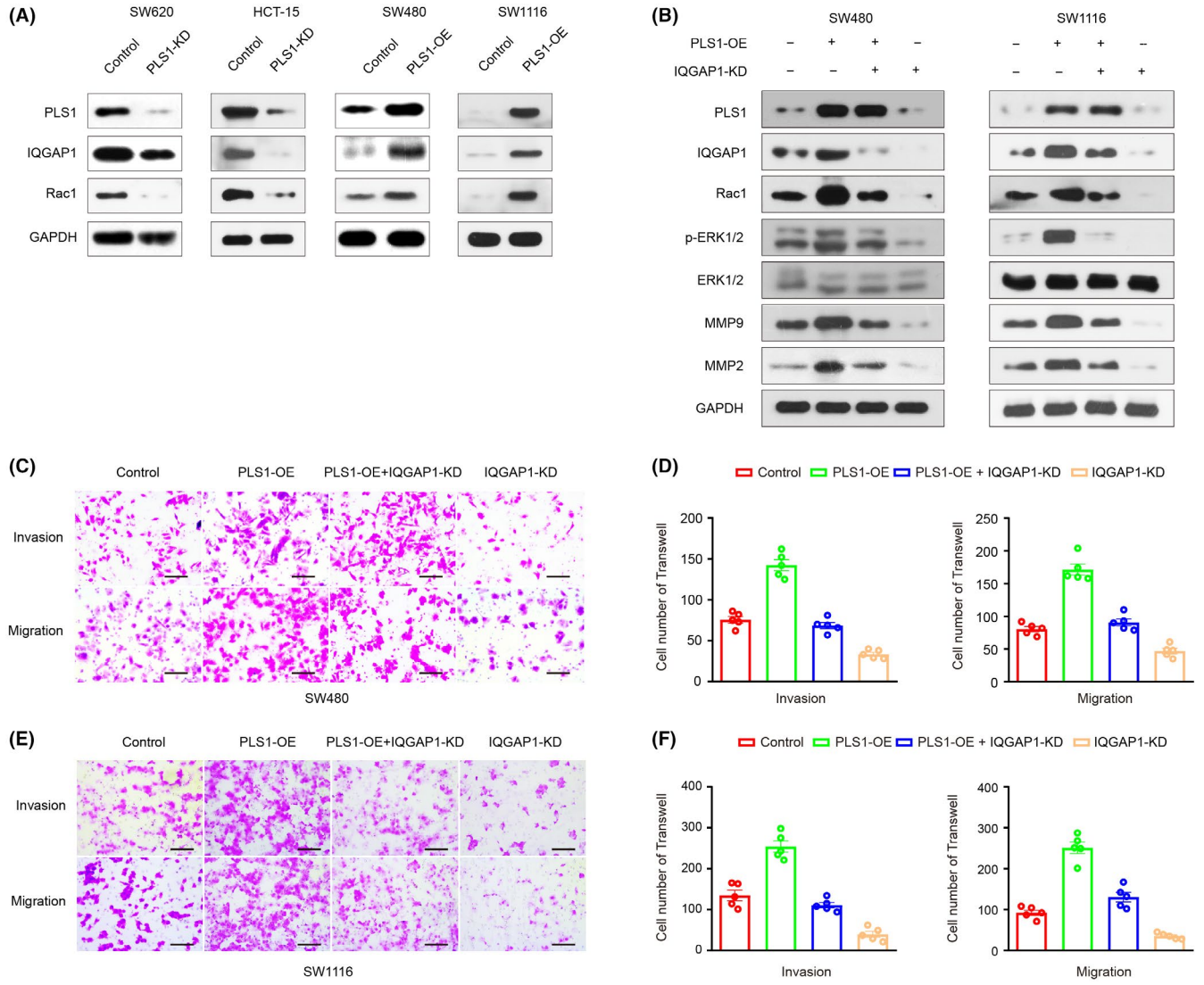

FIGURE 4 Plastin 1 (PLS1) promotes colorectal cancer cell invasion and migration through the IQGAP1-Rac1 pathway. A, Downregulation of PLS1 expression inhibited the expression of IQGAP1 and Rac1 in SW620 and HCT-15 cells, whereas upregulation of PLS1 elevated the expression of IQGAP1 and Rac1 in SW480 and SW1116 cells, as shown by western blot analysis. B, IQGAP1-specific siRNA partially reversed p-ERK1/2 upregulation in SW480 and SW1116 cells with PLS1 overexpression, as shown by western blot analysis. C, Invasion and migration capacity of SW480 cells using the transwell assay. Scale bars, 100 μm. D, Number of transwell cells was quantified to detect invasion. E, Invasion and migration capacity of SW1116 cells using the transwell assay. Scale bars, 100 μm. F, Number of Transwell cells was quantified to detect invasion. Error bars, means ± SD (n = 3 independent experiments)

upregulated in CRC tissues and that overexpression of IQGAP1 promotes CRC cell invasion in vitro. Another in vivo study reported that the cell growth, cell migration and tumorigenesis of CRC are affected through IQGAP1 activating the phosphorylation of ERK.[22] Thus, we presumed that the IQGAP1 pathway might be involved in stimulating the ERK activity induced by PLS1. Knockdown of PLS1 in SW620 and HCT-15 cells inhibited the expression of IQGAP1 and Rac1. Conversely, upregulation of PLS1 in SW480 and SW1116 cells elevated the protein levels of IQGAP1 and Rac1 (Figure 4A and Figure S4).

Through the recovery assay, we found that IQGAP1-specific siRNA could partially reverse the ERK1/2 activity and downstream MMP upregulation in SW480 and SW1116 cells in which PLS1 was overexpressed (Figure 4B). The invasion and migration ability of CRC cells was decreased by sh-IQGAP1, and PLS1 was overexpressed (Figure 4C-F). These results suggest that PLS1 might regulate ERK activity through the IQGAP1-Rac1 pathway in CRC cells.

3.6 | Plastin 1 is co–expressed with the IQGAP1-Rac1 pathway in colorectal cancer

To confirm whether the mechanism recognized in vitro exists in patients, we investigated the relationship of PLS1 and IQGAP1 in human CRC tissues. IHC staining of primary tumor tissues, which were resected from 135 CRC patients, with the anti–PLS1 and anti–IQGAP1 antibodies, was performed (Figure 5A). The mean values of

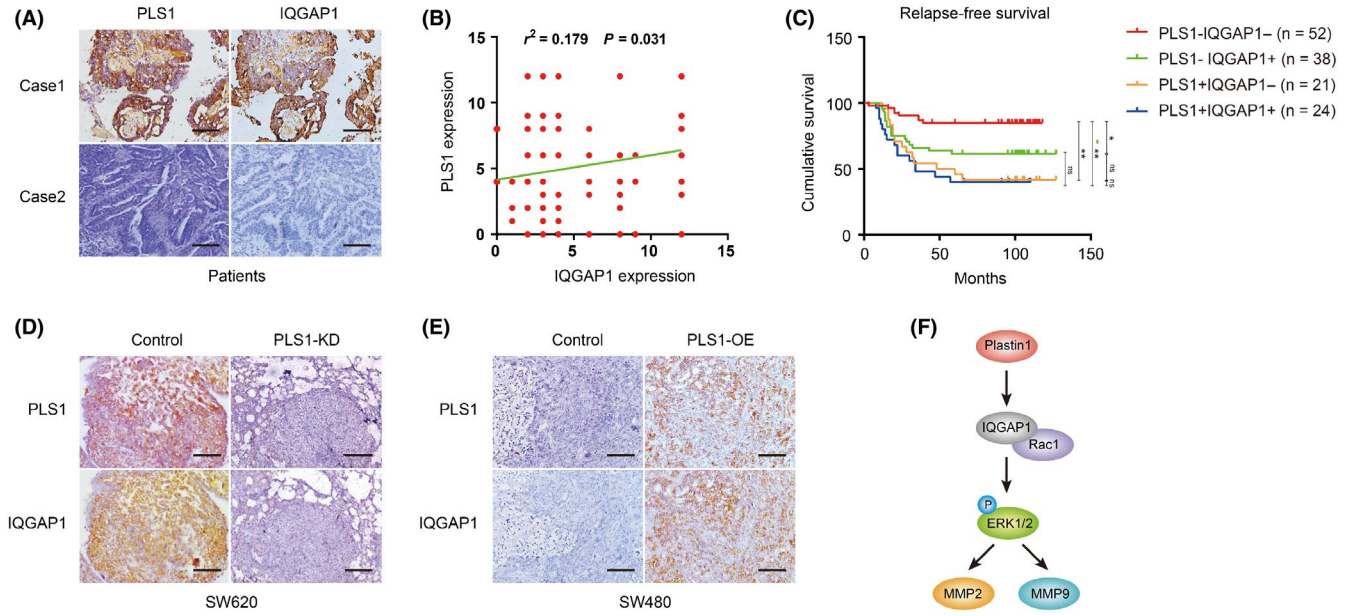

FIGURE 5 Plastin 1 (PLS1) is co–expressed with the IQGAP1-Rac1 pathway. A, Correlation between PLS1 and IQGAP1 levels in colorectal cancer by immunohistochemistry assay. B, Pearson's correlation between PLS1 expression and IQGAP1 in colorectal cancer tissue of patients. C, The Kaplan-Meier analysis showed that patients with simultaneous overexpression of PLS1 and IQGAP1 had the shortest relapse-free survival (RFS) times. Correlation between PLS1 and IQGAP1 levels in lung metastasis nodules (D) or liver metastasis nodules of mice injected with colorectal cancer cells was detected by immunohistochemistry assay (E). F, PLS1 might drive metastasis via the IQGAP1-Rac1 pathway. Scale bars, 100 μm; error bars, means ± SD (n = 3 independent experiments). $^*P < 0.05$; $^{**}P < 0.01$; not significant

scores were used as the criteria for expression levels of these two proteins. Statistical analysis revealed that higher expression of these two proteins was observed, and they were significantly correlated with each other in CRC tissues (Figure 5B). K-M analysis also showed that patients with simultaneous overexpression of PLS1 and IQGAP1 had the shortest disease-free survival time and overall survival time (Figure 5C and Figure S5). We next determined whether there was a close relationship between IQGAP1 and PLS1, but they did not show interaction with each other in the immunoprecipitation assay (Figure S6).

Furthermore, PLS1 expression was positively correlated with IQGAP1 expression in lung metastatic nodules from mice injected with SW620 cells (Figure 5D), which was the same as that of liver metastatic nodules from mice injected with SW480 cells (Figure 5E). Taken together, these results indicate that PLS1 promotes CRC cell metastasis partly by modulating the IQGAP1/Rac1/ERK signaling pathway (Figure 5F).

4 | DISCUSSION

Deregulated expression of plastins has been found in human malignant tumors. The role of PLS2 and PLS3 has been documented in CRC tissues.[23,24] However, there is little information about the behavior of PLS1 in tumors. PLS1 is a highly abundant protein in gastrointestinal tissues (https://www.proteinatlas.org/ENSG00000120756-PLS1/tissue),[25] but it has been overlooked in the past 10 years, even in gastrointestinal tumors.

Metastatic cancer cells use actin bundles to underpin protrusions that allow them to break away from a primary tumor and migrate to the surrounding tissue.[26-28] As a concatenate protein of F-actin, plastins are positively correlated with the migration and invasion of cancer cells.[6,29-31] The human PLS1 gene has been reported to be expressed in the intestine, kidney and cochlea,[4] but it has been overlooked in the past 10 years, even in gastrointestinal tumors.

Our data showed that PLS1 is highly expressed in CRC tissues and is correlated with poor prognosis. Through in vitro and in vivo experiments, PLS1 presented as an accelerator for invasion and migration of CRC cells and the liver or lung metastasis. This data suggests that PLS1 drives metastasis of CRC cells. The mechanisms of metastasis are very complicated, with the involvement of several molecules, and the specific role of PLS1 is unclear. In recent 20 years, MMP have emerged as crucial proteases that degrade the extracellular matrix (ECM) and pose an environment beneficial for the invasion or metastasis of cancer cells.[12] MMP9 is tightly associated with the metastasis of CRC and shows potential to be a prognostic biomarker.[18-20,32] As expected, MMP9 and MMP2 were regulated by PLS1, which depended on the activation of the ERK signal. This result reveals the targets and the downstream signal of PLS1; what happens upstream of ERKis unclear.

We have previously demonstrated that IQGAP1 knockdown significantly suppresses CRC cell migration and invasion in vitro.[25,25] Moreover, the cell growth, cell migration and tumorigenesis of CRC have been shown to be reinforced through the ERK pathway activated by SUMOylated IQGAP1.[33] IQGAP1 is a conserved modular protein organizing actin and microtubules in cell motility.[34] IQGAP1

binding to Rac1 and Cdc42 are necessary to assemble an actin-meshwork for building strong cell-cell adhesion,[35] which is also present in colon cells.[36] In addition, overexpression of IQGAP1 at the invasion front of CRC tissues demonstrates the invasive capability of cancer cells.[37] Therefore, we wondered whether the IQGAP1 signal is linked to PLS1. Consequently, PLS1 was found to upregulate IQGAP1 and Rac1 expression and result in the promotion of CRC cell metastasis. The positive association among PLS1, IQGAP1 and Rac1 was mirrored in CRC tissues of patients and the invaded lymph nodules from a mouse model as well. These data illustrate the signal transduction for CRC metastasis originating from PLS1.

Plastin 1 and IQGAP1 are not associated with each other, but the details connecting the two molecules and actin warrant further investigation. For instance, it would be significant to examine whether blocking the interaction between actin and PLS1 can inhibit CRC metastasis.

In summary, our study provides a better understanding of the molecular mechanisms of PLS1 involved in CRC metastasis. Furthermore, our results suggest a potential role of PLS1 as a biomarker of disease progression and prognosis in CRC patients.

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DISCLOSURE

The authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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