Specific protein 1(SP1) regulates epithelial-mesenchymal transition via lysyl oxidase-like 2(LOXL2) in pancreatic adenocarcinoma.

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Directed by Professor Joon Seong Park

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ABSTRACT

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Specific protein 1 (SP1) is with aggressive behavior, invasive clinical phenotype and poor clinical outcomes in various cancers. We studied whether SP1 exerts its effect on invasiveness and promotion of the epithelial-mesenchymal transition (EMT) by regulating lysyl oxidase-like 2 (LOXL2) in pancreatic cancer (PC) cell lines and analyzed the prognostic value of SP1 in patients with PC.

First, we verified the expression of SP1 in pancreatic adenocarcinoma (PDAC) cell lines. Then, we showed that silencing of SP1 in MIA Paca-2 cell induced significant decrease of cell invasion and migration.

We performed chromatin immunoprecipitation assays to confirm the binding of SP1 to LOXL2 promoter. In MIA Paca-2 cells, silencing of SP1 induced a reduction of LOXL2-expression, whereas LOXL2 silencing did not lead to a decrease in the expression of SP1. Transmigration and wound healing assays demonstrated the greatest decline in cell migration when both SP1 and LOXL2 were silenced.

Immunohistochemical analysis was performed in tissues from 62 patients with PDAC. Univariate and multivariate analyses of clinical data showed that tumor differentiation and co-overexpression of SP1 and LOXL2 were independent factors for disease-free survival.

In conclusion, our study demonstrated that SP1 modulates EMT and is involved in tumor invasion and migration of PC cells through the regulation of LOXL2.

Key words: specific-protein 1 (SP1), lysyl oxidase-like 2 (LOXL2), epithelial-mesenchymal transition (EMT), pancreatic adenocarcinoma

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I. INTRODUCTION

Pancreatic cancer (PC) is the 5th leading cause of cancer-related deaths in Korea[1], and is still one of the most aggressive and lethal malignancies. The poor prognosis is driven from early local invasion and high incidence of recurrence. Therefore, it is important to fully understand the mechanism of early invasion and epithelial-mesenchymal transition (EMT) in PC.

Lysyl oxidase-like 2 (LOXL2) is a member of the lysyl oxidase family, responsible for the stabilization of collagen and elastin fibers in the extracellular matrix[2]. Up-regulation of LOXL2 is associated with oncogenic stress response and tumorigenesis in pancreatic ductal adenocarcinoma (PDAC)[3]. Additionally, in PC cell lines, LOXL2 activates EMT-like processes which are associated with invasive and migratory properties[4].

Specific Protein 1 (SP1), identified as a promoter-specific binding factor essential for transcription of the SV40 major Immediate Eearly (IE) gene[5,6], has been regarded as a general transcription factor mainly required for transcription of

housekeeping genes[7]. Importantly, high SP1 levels in pancreatic neoplasm are correlated with aggressive behavior, invasive clinical phenotype, increased recurrence rates, and decreased survival[8-11]. Also, both SP1 and EMT markers levels are elevated in cancer cells[12].

SP1 has been indicated as one of the potential transcription factors involved in LOXL2 expression[13]. Therefore, here, we investigated the effect of SP1 on invasiveness and promotion of EMT in PC cells through LOXL2 regulation and evaluated the clinical impact of SP1 as a prognostic factor in patients with PC.

II. MATERIALS AND METHODS

1. Patients

Between November 2002 and May 2012, 84 patients underwent radical resection for PDAC at the Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. 22 patients were excluded due to poorly preserved tissue samples, incomplete medical records, or no follow-up, and a total of 62 patients were retrospectively reviewed. Patients were examined for follow-up every three months during the first year, and every six months afterwards. This study was approved by the Institutional Review Board of Gangnam Severance Hospital, Yonsei University, Seoul, Korea (3-2014-0153).

2. Immunohistochemical (IHC) Staining

Serial sections (5 μm) of each block were adhered to poly-L-lysine-covered slides and incubated at 62˚C for 60 minutes. After deparaffinization and rehydration, the sections were heated in a microwave containing a 10-mM citrate buffer (pH 6.0) solution for 15 minutes and stained. An anti-SP1 antibody (Thermofisher Scientific, Cleveland, Ohio, USA) and an anti-LOXL2 antibody (Abcam, Cambridge, UK) were used at a 1:1,000 dilution. Normal pancreas tissue present within the block and appropriate control tissues were used as positive controls.

IHC staining was categorized as negative, “1+”, “2+”, or “3+” in high-power fields (200x) according to the intensity of cytoplasmic staining. Positivity was assigned for the scores “2+” and “3+”. The staining intensity was assessed by two pathologists, blind to the clinical outcomes.

3. Cell culture

The PC cell lines MIA Paca-2, PANC-1, AsPC-1, and BxPC-3 were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and

grown in accordance with ATCC recommendations.

4. SP1 RNA interference

For gene knockdown of gene expression, the following small interfering RNA (siRNA) were purchased from Bioneer (Daejeon, Korea): siSP1 (sense: 5'–CA GAUACCAGACCUCUUCU-3', antisense: 5'-AGAAGAGGUCUGGUAUCUG -3'), siLOXL2 (sense: 5-CAGUCUAUUAUAGUCACAU-3', antisense: 5'-AU GUGACUAUAAUAGACUG-3'). Transfection was carried out using TransIT® -LT1 (Mirus Bio, Madinon, Wi, USA) or Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturers' instructions. Cells were harvested and processed 48-72 hours post-transfection.

5. Reverse transcription polymerase chain reaction (RT PCR)

RNA was extracted using Trizol (Invitrogen), and purified by RNA extraction kit (easy-spin Total RNA extraction kit, Intron, Korea). For RT PCR, retro-transcription used a one-step RT PCR kit (Intron) and a GeneAmp PCR System 9700 (Appied Biosystems, Foster City, CA, USA) according to the manufacturers' instructions.

The primers used for RT PCR were: SP1 (F:5'-CCATACCCCTTAACCCCG-3', and R:GAATTTTCACTAATGTTTCCCACC-3'); LOXL2 (F:5'-AACGAGG C GACCCTTGCAGC-3', and R:5'-GGGTGCGCTTGCGGTAGGTT-3'); Snail (F: 5'-AATCGGAAGCCTAACTACAGCGAG-3', and R:5'-CTTTCCCACTGTCC TCATCTGACA-3'); CDH1 (F:5'-GACGCGGACGATGATGTGAAC-3', and R:5'-TGTACGTGGTGGGATTGAAGA-3'); L1CAM (F:5'-GCCACCTGTCAT CACGGAAC-3', and R: 5'-GTCCAGCGGAACTGCACTTC-3') and GAPDH (F:5'-CGGGAAGCTTGTGATCAATGG-3', and R:5'-GGCAGTGATGGCATG GACTG-3'). The experiments were performed in triplicate and normalized to GAPDH.

6. Western blot analysis

Cell lysates were prepared in RIPA buffer (Sigma, St Louis, MO, USA) supplemented with protease inhibitors. Protein samples were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The membranes were blocked and probed with primary antibodies against SP1 (Thermofisher Scientific, Cleveland, Ohio, USA), LOXL2 (Abcam), Snail (Cell Signaling; Danvers, MA, USA), CDH1 (BD Biosciences; Sparks, MD, USA), L1CAM (Novus Biologicals, Littleton, CO, USA), N-cadherin (Invitrogen), Vimentin (Dako, Glostrup, Denmark), β-actin (Santa Cruz Biotechnology, USA) and γ-Tubulin(Sigma). The intensity of the western blot signals was quantified using the ImageJ software (NIH) and normalized to β-actin and γ-tubulin.

7. Wound healing assay

For wound healing assays, we used 24-well culture inserts (ibidi, Martinsried, Germany). Cells were transfected and 24 hours after, were counted: 70μl of a cell suspension at $3x10^5$ cells/ml, and were plated on each side of the insert. After 24 hours, the insert was removed to create a wound, and 0.5ml of growth medium were added. After 24 hours, the cells were washed three times with phosphate-buffered saline and incubated in growth medium for 24 hours. The scratch wounds were then visualized under a microscope (Axio Observer Z1; Zeiss, Jena, Germany).

8. Transmigration assay

For the transmigration assay, the upper side of 8.0μm Transwell® inserts (Corning Costar, NY, USA) was coated with Matrigel (BD Bioscience). After transfection, $1×10^5$ of MIA Paca-2 cells were re-suspended in serum-free medium and plated on the upper chambers. The lower chambers were filled with growth medium. After 24 hours, the cells on the lower surface of the membrane

were stained using a Diff-Quick staining kit (Sysmex, Kobe, Japan), and were counted under an inverted microscope. Values are expressed as mean cell numbers in five random fields of view (200x).

9. Chromatin Immunoprecipitation (ChIP) assay

ChIP assays were performed with the EpiTect ChIP Oneday kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. A total of 5×10^6 MIA Paca-2 cells were cross-linked with 1% formaldehyde at room temperature for 10 min. Sonication was performed on ice to get 200 to1000 bp DNA fragments. Fragments were immunoprecipitated with an anti-IgG antibody (Santa Cruz Biotechnology) and the anti-SP1 antibody. After reverse cross-linking and DNA isolation and purification, DNA from input (diluted 1:100) or immunoprecipitated samples were assayed by quantative PCR (qPCR), performed on a LightCycler®480 Real-Time PCR system (Roche, Diagnostics, Mannheim, Germany) using the SYBT Green I Mastermix (Applied Biosystems). Then, the products were separated by 2% agarose gel electrophoresis. The following primers were used: P1 (-898 to -778 F:5'-CAACCCTGACCCCAG CCTCT-3', and R: 5'-GCAA GGAAAGAGACACAGCA-3'), P2 (-878 to -758 F:5'-GTCCTTTACTCCACAGATAGATGGGT-3', and R:5'-GGGACAACTA TTTGGCTGCAG-3'), P3 (-75 9 to -639 F:5'-CTGCGCCAAATAGTTGTCCC -3', and R:5'-AGCAGGGCTTAGAGAGAAACG-3'), P4 (-568 to 448 F:5'-GG ACATCTGGGGGTCCTCT-3', and R:5'-GCGGATTTTGCATTTCACTCCA -3'), P5 (-210 to 90 F:5'-GTTGCCCAGAGCTCACTG-3', and R:5'-GGTTAC CCTACTGGCCTGG-3').

10. Statistical analysis

All statistical analyses were performed using the SPSS software, version 21.0 (SPSS Inc., Chicago, IL). Categorical variables were evaluated using chi-square or the Fisher's exact tests, and continuous variables were analyzed using the

Student's t-test. Survival curves were plotted using the Kaplan-Meier method, and differences in survival time among groups were assessed with the log-rank test. Disease-free survival (DFS) was defined as the time interval between the date of surgery and the date of recurrence or last follow-up. The Cox proportional hazards regression method was used to determine independent prognostic factors. A *P-value* lower than 0.05 was considered statistically significant.

III. RESULTS

1. Correlation between SP1 expression and EMT markers in PDAC.

The expression of SP1 was analyzed in the MIA Paca-2, PANC-1, AsPC-1, and BxPC-3 PC cell lines. SP1 was detected in all cell lines. Additionally, SP1 expression was positively correlated with the expression of EMT markers such as Snail and L1CAM (Figure 1.).

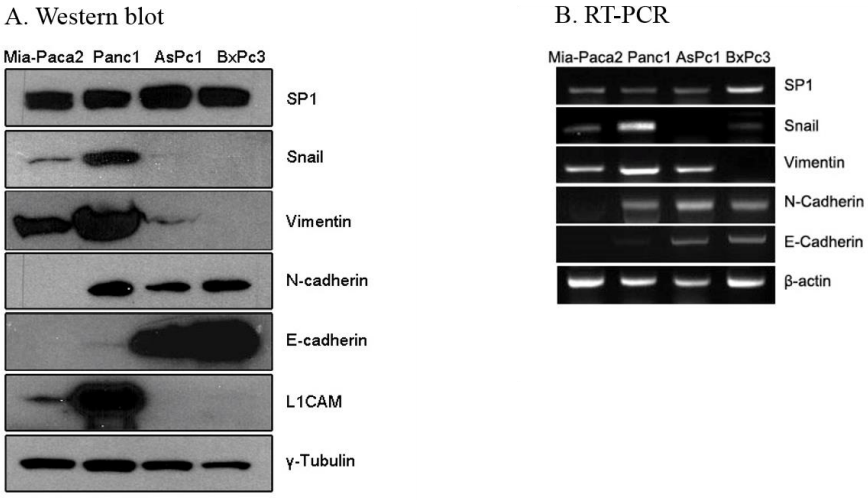
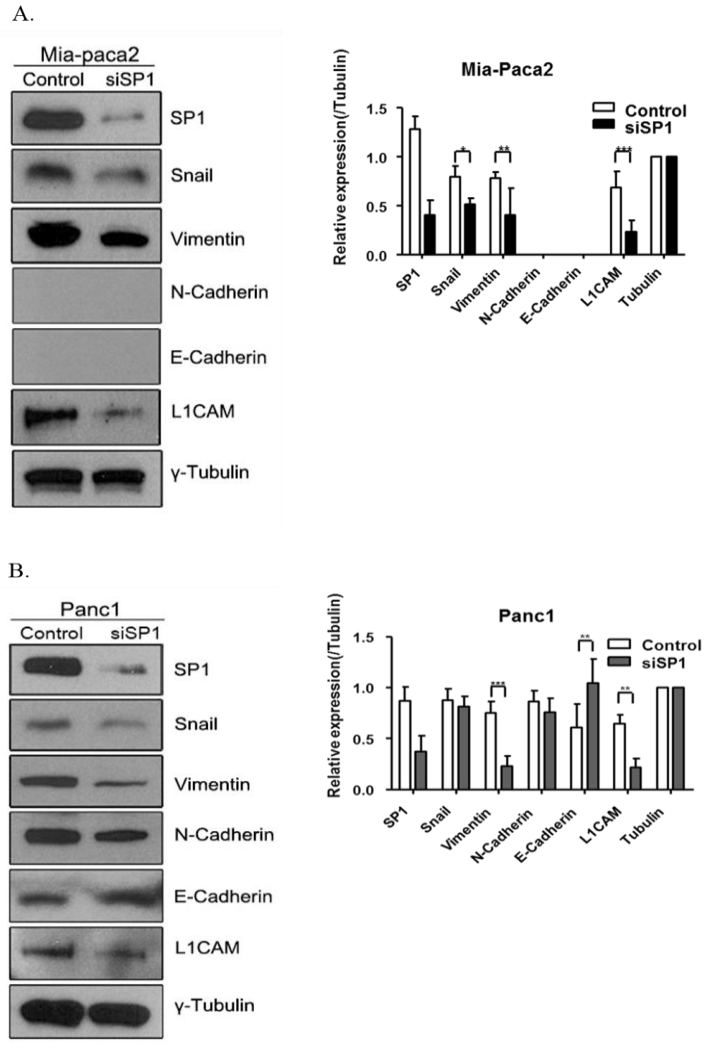


Figure 1. Expression of SP1 and EMT markers in PDAC cell lines.

A.B. Western blot analysis (A) and RT PCR (B) for the expression of SP1 and EMT markers in the indicated cell lines. γ-tubulin (A) and β-actin (B) were used for normalization.

To evaluate the effect of SP1 on the expression of EMT markers, we generated MIA Paca-2 and PANC-1 cells in which SP1 had been silenced. SP1-silenced cells exhibited significant reduction in the expression of Snail, important for EMT. Additionally, in Paca-2-siSP1, the expression of vimentin and L1CAM (mesenchymal markers) was reduced, whereas in PANC-1-siSP1, the expression of E-Cadherin (epithelial marker), was increased (Figure 2.)



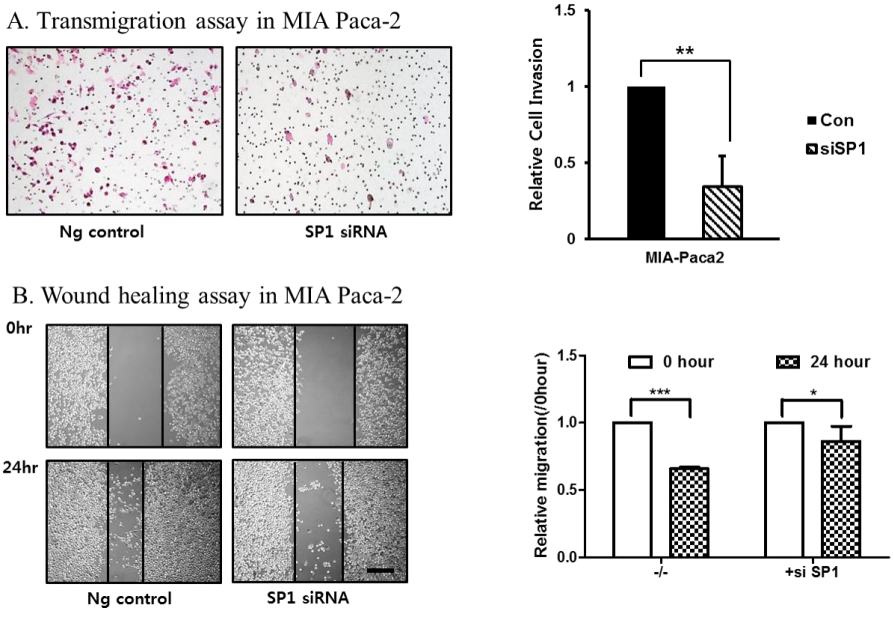
*: p<0.05, **: p<0.01, ***: p<0.00

Figure 2. SP1 affects the expression of EMT markers in PDAC cell lines.

A. Western blotting and densitometric analyses of the indicated proteins in MIA Paca-2 cells: silencing of SP1(siSP1) reduced SP1, Snail, Vimentin and L1CAM levels. **B.** Western blotting and densitometric analyses of the indicated proteins in PANC-1 cells: silencing of SP1(siSP1) reduced SP1 and Snail, and increased E-Cadherin. γ-tubulin was used for normalization.

2. Effects of SP1 expression on cell invasion and migration.

In transmigration assays, SP1-silenced MIA Paca-2 cells showed a statistically significant decrease of cell invasion (Figure 3A.). Moreover, in wound healing assays, SP1 silencing significantly decreased cell migration compared to control cells (Figure 3B.).



*: p<0.05, **: p<0.01, ***: p<0.001

Figure 3. Knockdown of SP1 inhibits the invasion and migration of PDAC cells.

A. Transmigration assay in MIA Paca-2 cells transfected with control or SP1 siRNAs: SP1 knockdown significantly inhibits cell invasion. **B.** Wound healing assay in MIA Paca-2 cells transfected with control or SP1 siRNAs: SP1 knockdown significantly decreased cell migration 24 hours after removal of the insert (see Materials and Methods for details).

3. SP1 regulates EMT through LOXL2.

Higher LOXL2 expression is associated with the invasiveness of PDAC and the lower survival rate of patients. As demonstrated in Figure 4A., LOXL-2 was detected in MIA Paca-2 and PANC-1 cells, in agreement with our previous study[4]. In MIA Paca-2, SP1 silencing induced a reduction of LOXL-2 expression, whereas LOXL-2 silencing did not affect SP1 levels (Figure 4B.). Additionally, ChIP assays with qPCR demonstrated the binding of SP1 to LOXL2 promoter (Figure 4C.).

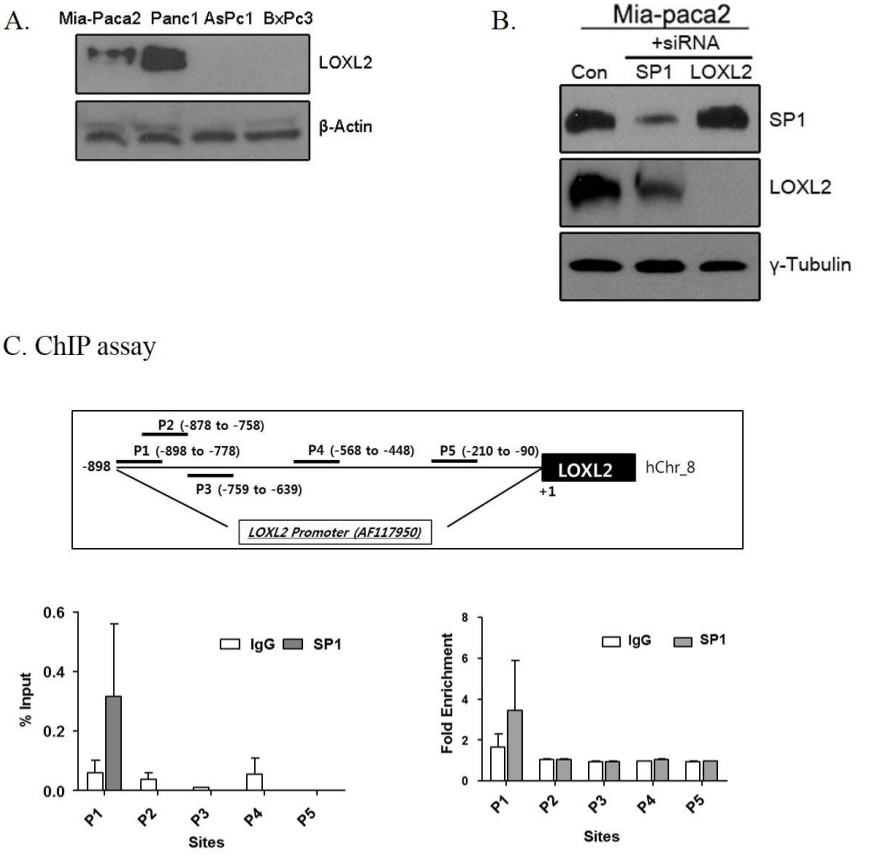
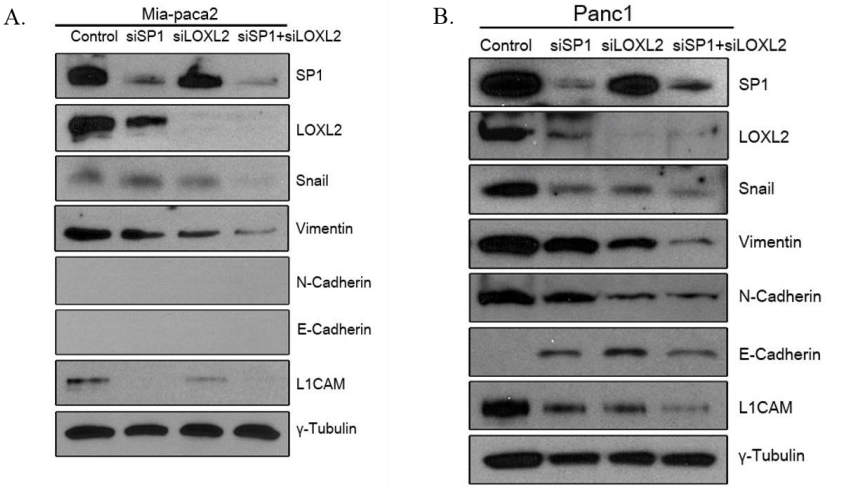
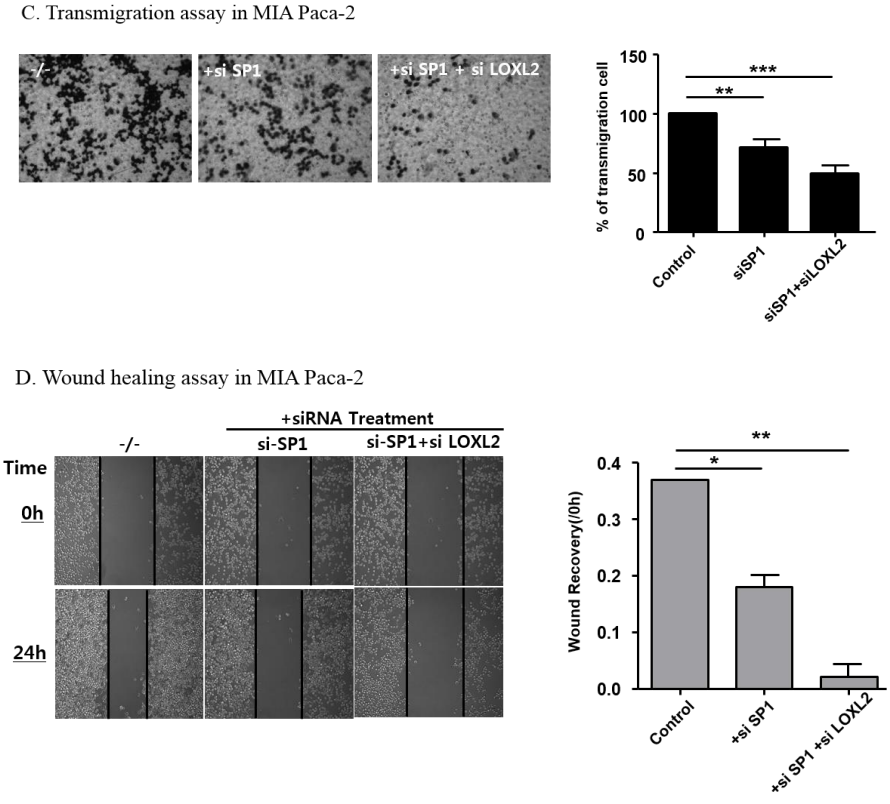


Figure 4. SP1 regulates the expression of EMT-related factor LOXL2.

A. LOXL2 expression in PDAC cell lines. **B:** Western blot analysis after silencing LOXL2 or SP1 in MIA Paca-2 cells: SP1 silencing induced a reduction of LOXL-2 expression, whereas LOXL-2 silencing could not lead to decrease the expression of SP1. **C.** ChIP assay. The scheme indicates LOXL2 promoter and the regions recognized by the primers used in the qPCR assays. These assays show that SP1 binds to the P1 region of LOXL2 promoter.

To investigate how SP1 regulates EMT through LOXL2, we performed additional silencing experiments. In MIA Paca-2 and PANC-1 cells, silencing of both factors induced a remarkable decrease of EMT marker levels, higher than that obtained upon silencing of either SP1 or LOXL2 (Figure 5A and B.). Additionally, transmigration and wound healing assays showed the greatest decline in cell migration when both factors were silenced (Figure 5C and D.).





*: p<0.05, **: p<0.01, ***: p<0.001

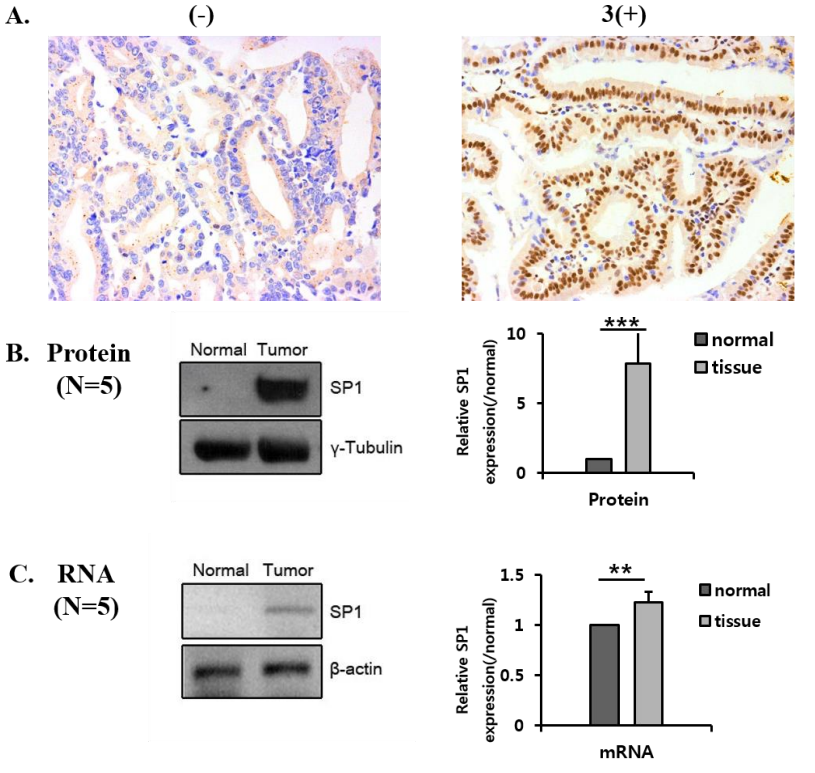
Figure 5. SP1 regulates EMT of PDAC cells through LOXL2.

A.B. Western blot analyses in MIA Paca-2 and PANC-1; Silencing of SP1 and LOXL2 induced a remarkable decrease of EMT markers as well as silencing of LOXL2 only. **C.** Transmigration assay in MIA Paca-2; Transfection of both SP1/LOXL2 siRNA induced most significant decrease of cell invasion compared to SP1-only silenced cells. **D.** Wound healing assay in MIA Paca-2; Transfection of both SP1/LOXL2 siRNA decreased cell migration more significantly compared to SP1-only silenced cells after 24 hours.

4. SP1 and LOXL2 expression in human samples.

Next, we investigated SP1 and LOXL2 expression in samples from 62 patients (Figure 6.). There was no significant difference among patients according to the expression of SP1/LOXL2 (Table 1.).

Multivariate analyses indicated that tumor differentiation and high expression of both SP1 and LOXL2 were independent poor prognostic factors for DFS (Table 2.). Additionally, Kaplan Meier survival analyses showed that the patients with high expression of both SP1 and LOXL2 had lower DFS compared to other groups with clinical significance (Figure 7.).



*: p<0.05, **: p<0.01, ***: p<0.001

Figure 6. Expression of SP1 in human tissues.

A. Immunohistochemical analysis of SP1. **B.** Western blot analysis of SP1 protein expression in normal and tumor tissues (N=5). The graph indicates the quantification of the results. **C.** RT PCR analysis of SP1 mRNA expression in normal and tumor tissues (N=5). The graph indicates the quantification of the results.

Table 1. Patient characteristics according to the expression of SP1 and LOXL2.

Factors	N=62	SP1			LOXL2		
		Negative (n=38)	Positive (n=24)	*P*	Negative (n=14)	Positive (n=48)	*P*
Sex				0.434			0.368
Male	28	19(50.0%)	9(37.5%)		8(57.1%)	20(41.7%)	
Female	34	19(50.0%)	15(62.5%)		6(42.9%)	28(58.3%)	
Age				0.311			0.700
≤60	11	5(13.2%)	6(25.0%)		3(21.4%)	8(16.7%)	
>60	51	33(86.8%)	18(75.0%)		11(78.6%)	40(83.3%)	
Operations				1.000			0.315
PD	44	27(71.1%)	17(70.8%)		8(57.1%)	36(75.0%)	
Others	15	11(28.9%)	7(29.2%)		6(42.9%)	12(25.0%)	
Differentiation				0.505			1.000
Well	11	8(21.1%)	3(12.5%)		2(14.3%)	9(18.8%)	
Mod. to Poor	51	30(78.9%)	21(87.5%)		12(85.7%)	39(81.3%)	
Tumor size (mean±SD, cm)		3.20±1.32	3.18±1.36	0.955	3.26±1.03	3.16±1.40	0.805
Invasion depth				0.736			1.000
T1/2	51	32(84.2%)	19(79.2%)		12(85.7%)	39(81.3%)	
T3/4	11	6(15.8%)	5(20.8%)		2(14.3%)	9(18.8%)	
LN metastasis				1.000			0.519
Negative	19	12(31.6%)	7(29.2%)		3(21.4%)	16(33.3%)	
Positive	43	26(68.4%)	17(70.8%)		11(78.6%)	32(66.7%)	
PNI				0.764			1.000
Negative	15	10(26.3%)	5(20.8%)		3(21.4%)	12(25.0%)	
Positive	47	28(73.7%)	19(79.2%)		11(78.6%)	36(75.0%)	
LVI				0.434			1.000
Negative	31	21(55.3%)	10(41.7%)		7(50.0%)	24(50.0%)	
Positive	31	17(44.7%)	14(58.3%)		7(50.0%)	24(50.0%)	

Abbreviation: N, number; PD, pancreaticoduodenectomy; Mod., moderate; LN, lymph node; PNI, perineural invasion; LVI, lymphovascular invasion.

Table 2. Univariate and multivariate analyses of the relationship between DFS and clinicopathologic variables by Cox regression hazard model.

Factors	N	DFS	Univariate analysis			Multivariate analysis		
		median(range)	HR	95% CI	*P*	HR	95% CI	*P*
Differentiation								
Well	11	16.1(5.8-87.4)	1			1		
Mod. to Poor	51	8.3(1.1-71.4)	3.050	1.365-6.817	**0.007**	3.474	1.528-7.899	**0.003**
Invasion depth								
T1/2	51	10.3(1.1-87.4)	1					
T3/4	11	11.8(3.5-69.7)	1.301	0.653-2.592	0.455			
LN metastasis								
Negative	19	8.3(1.1-69.5)	1					
Positive	43	11.0(1.5-87.4)	0.855	0.483-1.512	0.590			
PNI								
Negative	15	11.3(1.1-71.4)	1					
Positive	47	9.2(3.5-87.4)	1.071	0.501-1.741	0.830			
LVI								
Negative	31	12.7(1.1-69.7)	1					
Positive	31	9.4(1.5-87.4)	1.033	0.610-1.749	0.903			
SP1 status								
Negative	38	12.7(1.1-87.4)	1					
Positive	24	8.1(1.5-69.7)	1.270	0.742-2.176	0.384			
LOXL2 status								
Negative	14	13.2(7.0-71.4)	1					
Positive	48	8.3(1.1-87.4)	1.516	0.799-2.875	0.203			
SP1/LOXL2 status								
All others	45	12.7(1.1-87.4)	1			1		
SP1(+)/LOXL2(+)	17	7.1(1.5-56.0)	1.802	1.001-3.244	**0.039**	2.151	1.170-3.953	**0.014**

Abbreviation: N, number; DFS, disease-free survival; Mod., moderate; LN, lymph node; PNI, perineural invasion; LVI, lymphovascular invasion.

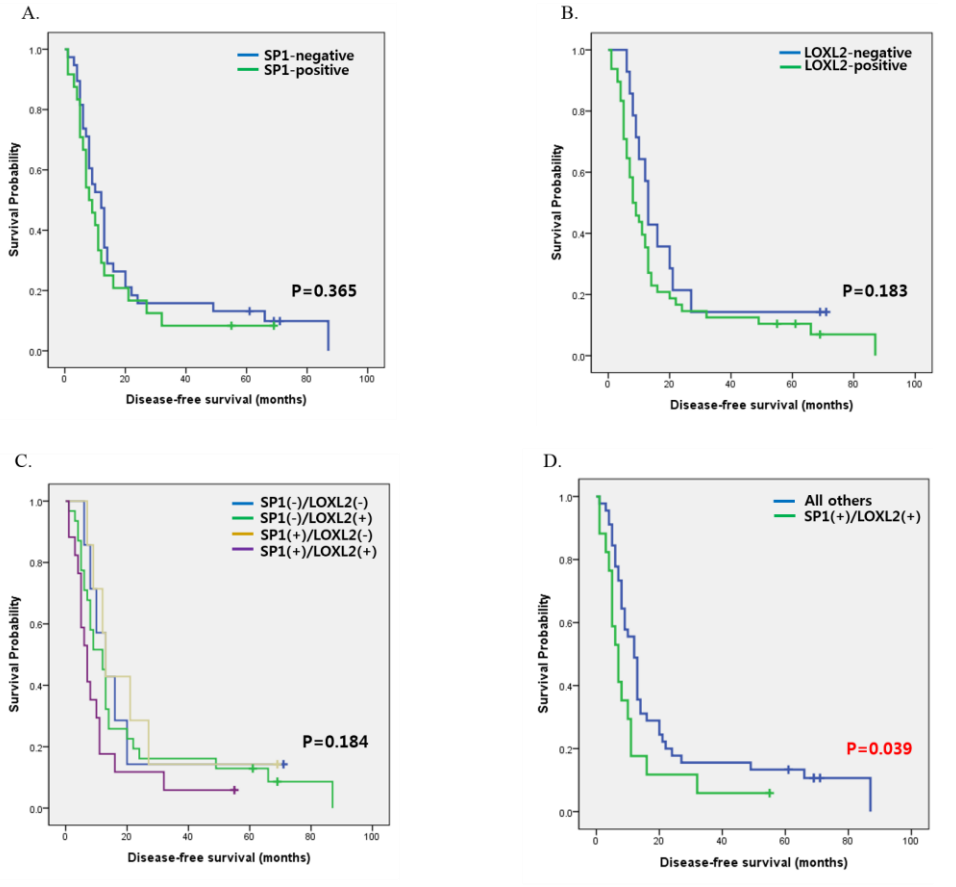


Figure 7. Disease-free survival analyses according to the expression of SP1 and LOXL2.

A.B. 5-year DFS curves according to the expression of SP1 or LOXL2: no significant differences were found according to the expression of SP1 or LOXL2. **C.** 5-year DFS curves according to the co-expression of two factors: there was no significant difference in 5-year DFS rates among the four indicated groups. **D.** 5-year DFS curves according to the expression of both SP1 and LOXL2 in the SP1(+)/LOXL2(+) vs. the SP1(-)/LOXL2(-), SP1(-)/LOXL2(+), and SP1(+)/ LOXL2(-) groups. The group with SP1(+)/LOXL2(+) showed significantly lower DFS compared to all others.

IV. DISCUSSION

PDAC is characterized by extensive stromal and matrix deposition (*demoplasia),* which generates high levels of stress in the tumors and compression of the vasculature, and promotes a hypoxic, nutrient-poor environment[14]. This poor conditions cause endoplasmic reticulum(ER) stress inducing the cell initiates unfolded or incorrectly folded protein production for adaptation[15-17].

SP1 is a highly regulated transcription factor that plays a critical role for the regulation of genes involved in cancer progression[18]. Earlier studies demonstrated SP1 overexpression in various cancers including sarcoma, and adenocarcinomas in colon or stomach[8-10,19-21], whereas minimal or no expression of SP1 was detected in normal differentiated cells. Similarly, SP1 is overexpressed in PC[11,12,15]. It has been reported that downregulation of SP1 interferes with recovery from ER stress, and the activation of unfolded protein response in PDAC[15].

The overexpression of LOXL2 in PDAC cells enhances EMT by inducing cell migration and invasion[4]. Additionally, LOXL2 is one of the most specifically and highly expressed genes in PC, therefore, researchers tried to detect higher regulation factor of LOXL2. One recent study reported that the promoter of LOXL2 contains binding sites for several transcription factors including SP1[13]. Therefore, we investigated whether SP1 regulates ENT through LOXL2. We found that SP1 binds to LOXL2 promoter, and regulates the expression of EMT markers by regulating LOXL2 expression.

Our clinical data demonstrated that the overexpression of SP1 and LOXL2 indicates poor prognosis in patients with PC. However, the overexpression of either SP1 or LOXL2 did not significantly correlates with poor outcome. One explanation is that SP1 regulates the expression of heterogenous target proteins[12]. Therefore the overexpression of SP1 may not always suggest advanced tumor or lymph node metastases. Moreover, the lack of correlation in our clinical data sets

may be due to the relatively small number of patients in this study, and bias on interpretation of immunohistochemistry.

Nevertheless, this study indicates that SP1 may serve as a therapeutic target for selected patients with PC, especially if silenced together with LOXL2. More thorough comprehension of SP1 activities is necessary before the use of SP1 as a target for PC treatment.

V. CONCLUSION

Our study demonstrated that SP1 modulates EMT, and is involved in invasion and migration of PC cells by regulating LOXL2. In clinical data, the co-overexpression of SP1 and LOXL2 significantly correlates with poor clinical outcome in patients with PC.

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ABSTRACT(IN KOREAN)

췌장암에서 LOXL2를 통한 SP1의 상피-간엽이행의 조절

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김 임 경

Specific protein 1 (SP1)은 다양한 암종에서 침습적인 임상양상 및 불량한 예후와 관련된 것으로 알려져 있다. 저자들은 이번 연구를 통하여 SP1이 이미 췌장암에서 전이와 침습에 관련하는 인자로 알려진 lysyl oxidase-like 2 (LOXL2)를 조절함으로써 상피-간엽이행을 조장하고 췌장암의 침습을 용이하게 하는데 어떠한 역할을 하는지 알아보고, 이를 임상자료를 통하여 확인해 보고자 하였다.

췌장암 세포주에서 SP1의 과발현을 확인한 뒤, MIA Paca-2에서 SP1을 억제한(사일런싱, silencing) 결과, 유출 분석(transmigration assay)에서 세포의 침습도가 통계학적으로 유의하게 감소하였다. 또한, 상처치유분석(wound healing assay)에서도 SP1의 사일런싱이 24시간 후 세포 이동을 현저하게 감소시키는 것을 확인하였다.

SP1이 LOXL2를 조절하는 전사인자로서 역할을 하는 것을 확인하기 위하여 시행한 염색질 면역침강반응검사(chromatin immunoprecipitation assay) 상 LOXL2에서 SP1의 결합자리를 발견하였다. 또한, MIA Paca-2에서 SP1의 사일런싱이 LOXL2의 발현을 억제하였으나, LOXL2의 사일런싱은 SP1의 발현을 억제하지 못하여 SP1이 LOXL2의 우위 신호체계임을 알 수 있었다. SP1과 LOXL2를 모두 사일런싱한 경우에서, 각각을

사일런싱한 것과 비교하여 세포 이동의 가장 극적인 감소를 보였다.

실험결과를 바탕으로, 62명의 췌장암 환자 조직에서 면역염색을 시행하였다. 단변량 및 다변량 분석에서, 암세포의 분화도 및 SP1과 LOXL2의 동시과발현(co-overexpression)이 무병생존률을 예측하는 독립적인 인자로 확인되었다.

본 연구를 통하여 저자들은 SP1이 상피-간엽이행을 도모하고, LOXL2를 조절함으로써 암세포의 침습 및 전이에 참여하는 것을 알 수 있었다.

핵심되는 말: SP1, LOXL2, 상피-간엽이행, 췌장암