Novel gastric cancer stem cell-related marker, LINGO2 enhances migration and invasion in gastric cancer cells associated with epithelial-mesenchymal transition

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Directed by Professor Si Young Song

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<ABSTRACT>

Novel gastric cancer stem cell-related marker, LINGO2 enhances migration and invasion in gastric cancer cells associated with epithelial-mesenchymal transition

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(Directed by Professor Si Young Song)

Background: Cancer stem cells (CSCs) are a small subpopulation of cancer cells with the capacity for self-renewal, tumor initiation and drug resistance, similar to normal stem cells. CSCs are suggested to be critical in cancer invasion and metastasis related with the epithelial-mesenchymal transition (EMT).

Aim: We investigated the function of the novel gastric CSC-related marker, LINGO2 (leucine-rich repeat and immunoglobulin-like domain-containing nogo receptor-interacting protein 2 precursor) by comparing DNA microarray data for gastric cancer tumor sphere and adherent cells.

Methods: DNA microarray results from gastric cancer sphere and adherent cells were analyzed for cancer stem cell-related markers. LINGO2 was upregulated in both sphere-forming gastric cancer cell lines. LINGO2 expression was confirmed by RT-PCR, western blots of gastric cancer cells and immunohistochemistry of a human gastric cancer tissue microarray. To determine LINGO2 functions, gastric cancer sphere cells were sorted by LINGO2 expression into LINGO2-high and LINGO2-low cells. And, we performed targeted knockdown using shRNA in SNU484 gastric cancer cell lines.

Results: LINGO2 was expressed in gastric cancer sphere cells and most gastric cancer cell lines. Migration ability increased in cells with LINGO2-high compared to LINGO2-low expression. LINGO2 shRNA-knockdown cells also had decreased migration and invasion in SNU484 gastric cancer cells. Expression of mesenchymal markers N-cadherin, vimentin, and matrix

metalloproteinases were significantly inhibited in LINGO2 knockdown cells. LINGO2 knockdown reduced CD44 expression and AKT and MEK/ERK phosphorylation. Clinically, LINGO2 expression correlated positively with lymph node invasion in human gastric cancer tissues.

Conclusion: These results suggested that the novel gastric CSC-related marker LINGO2, which is associated with the EMT pathway, enhanced migration and invasion in gastric cancer cells. LINGO2 is a potential therapeutic target for gastric cancer.

Keywords: cancer stem cell; gastric cancer; LINGO2; epithelial-mesenchymal transition; invasion; migration

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I. INTRODUCTION

Gastric cancer is the fourth most common malignant tumor worldwide and the second leading cause of cancer death.[1] Although gastric cancer mortality has recently declined due to early detection by endoscopy, prognosis of gastric cancer is still poor with a 5-year survival rate of 20%.[2]

Cancer stem cells (CSCs) are a subpopulation in tumors with the capacity for self-renewal. CSCs are typically less than 5% of total tumor cells[3] but are critical in cancer initiation, invasion, metastasis and drug resistance.[4,5] Recently, CSC therapy is a new strategy of targeted therapy that focuses on overcoming the limitations of current treatments. Although traditional cancer therapies such as conventional chemotherapy and radiotherapy might be effective at destroying the majority of tumors, many cancers recur because of resistance to these therapies, which are ineffective against CSCs. Current cancer therapies showed a high incidence of adverse effects because of the lack of specificity to cancerous cells. CSCs express protective drug-transport mechanisms that efflux cytotoxic chemicals from cells[6,7] through ATP-binding cassette (ABC) and multidrug resistance (MDR)

transporters and activation of DNA repair mechanisms.[8-10]

Recent studies show that cancer cells in the epithelial-mesenchymal transition (EMT) share many properties with CSCs.[11-14] EMT was first observed and defined by Hay in the late 1960s.[15] The EMT is an essential process for normal embryonic development and reflects a reversible embryonic program. However, aberrant activation of EMT disturbs normal epithelial homeostasis, inducing pathologic alterations such as fibrosis, cancer cell invasion and metastasis to distant secondary organs.[16-18] EMT is the process by which epithelial cells are converted to mesenchymal cells with phenotypic changes such as loss of cell-cell adhesion, loss of cellular polarity and enhanced migration and invasion.[2] Epithelial cells are tightly associated with neighboring cells through E-cadherin-containing adherent junctions. Carcinomas derived from epithelial cells are the most prevalent (~90%) malignancies in humans[19,20] and are related to cancer morbidity and mortality. Cancer cells break intracellular junctions to migrate and invade stromal tissues during early cancer metastasis.[2] EMT is thought be the initial and most important step in the cancer metastasis cascade.

Gastric CSCs might be associated with EMT. Gastric CSCs were first found by Takaishi *et al.* in experiments showing that CD44-positive gastric cancer cells initiate tumors and cancer recurrence after treatment.[21] CD44-positive gastric cancer cells have stem cell properties and increased resistance to traditional chemotherapies and radiotherapies.[21] CD44 expression was significantly associated with expression of EMT-related molecules E-cadherin, vimentin, Snail-1, and ZEB-1 in gastric cancer patients by Ryu *et al.*[22]

We found the novel gastric CSC-related marker LINGO2 (leucine-rich repeat and immunoglobulin-like domain-containing nogo receptor-interacting protein 2 precursor) using DNA microarray results. The LINGO2 gene is on chromosome 9 at 9p21.2. This membrane protein is 606 amino acids and 68kDa. LINGO2 is a human paralog of LINGO1, with protein identity of 61%.[23] LINGO1 and LINGO2 are associated with an essential tremor and Parkinson's disease.[24,25] However, the function of LINGO2 is unknown.

In this study, LINGO2 knockdown cells established using shRNA to determine the LINGO2 functions. LINGO2 was found to enhance migration and invasion in gastric cancer cells and to be

highly expressed in patients with advanced lymph node invasion from human gastric cancer tissues. LINGO2 appeared to be related to the EMT pathway and could be a new therapeutic target for gastric cancer.

II. MATERIALS AND METHODS

1. Cell Culture

Human gastric cancer cell lines AGS, N87, SNU1, SNU5, SNU16, and NIH-3T3 mouse fibroblast cells were from the American Type Culture Collection (ATCC, Manassas, VA, USA). SNU484, SNU601, SNU638, and SNU668 cells were from the Korea Cell Line Bank (KCLB, Seoul, Korea). All cells were grown in RPMI1640 (Invitrogen Gibco, Grand Island, NY, USA) with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA), and kept in a humidified incubator with 5% CO_2 at 37℃.

2. Sphere Formation Assay

Cells were trypsinized and single-cell suspensions were confirmed microscopically, washed with phosphate-buffered saline (PBS), and resuspended. Cells (1000 cells/mL) were cultured in serum-free medium supplemented with 10 ng/mL epidermal growth factor (R&D Systems Inc., Minneapolis, MN, USA), 10 ng/mL basic fibroblast growth factor (R&D Systems Inc.), 1x insulin-transferring selenium (Invitrogen), 0.5% bovine serum albumin (Invitrogen) and 0.5% FBS. Single cells were seeded in 6-well ultralow attachment culture plates (Corning Inc., Corning, NY, USA). Adherent cultured cells, as a control, were seeded in culture dishes (Nalgene Nunc Intl, Rochester, NY, USA) with sphere-formation medium. After 7 days, cells were collected and dissociated with Accutase (Sigma-Aldrich, St. Louis, MO, USA).

3. Microarray Analysis

Total RNA was extracted from adherent and sphere cells from N87 and SNU484 gastric cancer cell lines using

RNeasy Miniprep kits (Qiagen, Valencia, CA, USA). RNA was quantitatively analyzed using a ND-1000 NanoDrop spectrophotometer (NanoDrop Technologies, Inc., DE, USA) and qualitatively analyzed on RNA 6000 nanochips (Agilent Technologies, Santa Clara, CA, USA) using an Agilent 2100 bioanalyzer (Agilent Technologies). Microarray experiments were according to manufacturer's protocol. Microarray analysis was performed by Digital Genomics, Inc. (Seoul, Korea). Double-stranded cDNA was prepared using 6 μg of total RNA labeled with biotin (IVT labeling kit; Affymetrix), followed by amplification. Labeled cDNA was fragmented and hybridized to Affymetrix GeneChip human genome U133 plus 2.0 high-density oligonucleotide arrays (Affymetrix, Santa Clara, CA, USA). Chips were washed with a Genechip Fluidics station 450 (Affymetrix) and scanned using a Genechip Array Scanner 3000 7G (Affymetrix). Expression data were generated using Affymetrix Expression Console software version 1.1 and normalized using MAS5 (Affymetrix Microarray Suite 5) algorithm. Expression intensity data in raw.cel files were normalized by the MAS5 algorithm to reduce noise. Probe sets that were not called "present" by MAS5 detection in 50% or more samples in at least one sample group were filtered out. A paired *t*-test was performed with MAS5 expression data to determine if genes were differentially expressed between two groups. Genes with more than 1.5-fold change were considered. Chip assays were repeated twice for each independent total RNA sample.

4. Semiquantitative Reverse-Transcription Polymerase Chain Reaction

Total RNA was extracted for reverse-transcription polymerase chain reaction (RT-PCR) using RNAeasy Mini kits (Qiagen), and complementary single-strand DNA was synthesized using a Superscript II system (Invitrogen) according to the manufacturers' protocols. Stemness-related molecules Jagged2, Notch3, Hes1, Ihh, Smo, Gli1, Fzd7, β-catenin, Nanog, Oct4 and PTEN were evaluated in gastric cancer sphere and control cells. β-actin (*ACTB*) was used as a reference gene. All of the primers are listed in Table 1.

Table 1. Primer sequences used for RT-PCR

Gene	Sense	Antisense
Notch3	ATGGTGGGAACTAAACACAGCT	ATGACCCTGGAGGAAGCACA
Jagged2	GTGGATGTCGACCTTTGTGA	GGCAGTCGTCAATGTTCTCA

Hes1	GTGCTGTCTGGATGCGGAGT	GAACACTCACACTCAAAGCCC
Ihh	CCTGAACTCGCTGGCTATCT	AATACACCCAGTCAAAGCCG
Gli1	AGAGTCCAGGGGGTTACATA	AGAGTCCAGGGGGTTACATA
Smo	GAATCGCTACCCTGCTGTTA	TGAGCAGGTGGAAGTAGGAG
Nanog	ACTGTCTCTCCTCTTCCTTCCT	AGAGTAAAGGCTGGGGTAGGTA
Oct4	GTGGAGGAAGCTGACAACAA	AGCAGCCTCAAAATCCTCTC
PTEN	GGACGAACTGGTGTAATGAT	CAGACCACAAACTGAGGATT
FZD7	CCAACGGCCTGATGTACTTT	GCCATGCCGAAGAAGTAGAG
β-catenin	GTATGAGTGGGAACAGGGATTT	CCTGGTCCTCGTCATTTAGC
β –actin	GGCATCCTCACCCTGAAGTA	GGGGTGTTGAAGGTCTCAAA
LINGO2	TTGCAAATATTGGCGTTCTG	TGATGCAAGGCTTTAACAAATG

5. Western Blots

Cells were lysed in lysis buffer containing 70 mM β-glycerophosphate (pH 7.2), 0.6 mM Na vanadate, 2 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 0.5% Triton X-100, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), and 1x complete protease inhibitor (Roche Applied Science, Nutley, NJ, USA). Protein (20 μg) was resolved on SDS-polyacrylamide gels and transferred to polyvinylidenedifluoride (PVDF) membranes (Immobilon-P, Millipore, Bedford, MA, USA). Membranes were blocked in 5% (w/v) skim milk and probed with primary antibodies: mouse monoclonal anti-LINGO2 (LifeSpan BioSciences, Inc., Seattle, WA, USA), mouse monoclonal anti-N-cadherin, rabbit polyclonal anti-αSMA, rabbit monoclonal anti-MMP9 (Abcam plc, Cambridge, UK), rabbit polyclonal anti-Oct4, rabbit polyclonal anti-phospho-GSK3, rabbit polyclonal anti-phospho-AKT, rabbit polyclonal anti-phospho-MEK, rabbit polyclonal anti-phospho-ERK (Cell Signaling Technology, Inc., Danvers, MA, USA), rabbit polyclonal anti-twist, rabbit polyclonal anti-E-cadherin, rabbit polyclonal anti-occludin, mouse monoclonal anti-PTEN, rabbit polyclonal anti-Gli1, rabbit polyclonal anti-Hey1, rabbit polyclonal anti-AKT, rabbit polyclonal anti-MEK, goat polyclonal anti-ERK, mouse monoclonal anti-

MMP1 and mouse monoclonal anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Afterwards, each membrane was washed with mixture of Tris-buffered saline and Tween 20 (TBST) buffer and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Santa Cruz Biotechnology) for 1 hour. Immunoblots were developed with West Pico Chemoiluminescent substrate (Thermo Scientific, Rockford, IL, USA). Quantitative results of protein expression determined by density measurement using NIH ImageJ software.

6. LINGO2 Immunohistochemistry

A total of 113 human gastric cancer tissues from surgical resections at Severance Hospital, Yonsei University College of Medicine were used. Gastric cancer tissue samples were embedded in paraffin and stained with hematoxylin and eosin (H & E). After pathological evaluation, tumor tissues were arrayed into new paraffin blocks for tissue microarrays (TMAs). The Ethical Committee for the Clinical Research of the Institutional Review Board of Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, approved this study protocol. TMA sections were deparaffinized in xylene and rehydrated in graded alcohol. Endogenous peroxidase activity was blocked with 0.3% (v/v) hydrogen peroxide in methanol. Antigen retrieval was performed by microwaving the slides in sodium citrate buffer (0.01M, pH 6.0) for 3 minutes. To block nonspecific staining, sections were incubated with 10% (v/v) normal donkey serum for 1 hour and incubated with mouse monoclonal anti-LINGO2 (1:500, LifeSpan BioSciences, Inc.) overnight at 4℃. Subsequent reaction used Envision kits (DakoCytomation California, Inc., Carpinteria, CA, USA) according to the manufacturer's instructions. Immunoreactions were developed with DAKO Liquid DAB+ substrate-chromogensystem and counterstained with Harris hematoxylin (Sigma-Aldrich). Immunoreactivity was scored as percentage of LINGO2-positive tumor cells: no expression, 0; <20%, 1+; 20-50%, 2+; and >50%, 3+.

7. Cell Sorting by LINGO2 Expression

Cells were dissociated with Accutase (Sigma-Aldrich) and resuspended in Hank's balanced salt solution (HBSS) with 2% FBS at 10^6 per 100 μL. Cells were stained with anti-LINGO2 (Lifespan Biosciences, Inc.) or purified mouse IgG2a, κ isotype (BD Biosciences PharMingen, San Diego, CA, USA) as a control for 20 minutes on ice and washed twice with HBSS/2% FBS. Secondary antibody phycoerythrin goat anti-mouse IgG (BD Biosciences PharMingen), was added to cells resuspended in HBSS/2% FBS followed by a 20 minutes incubation on ice. FACSAria II (BD Immunocytochemistry System, Franklin Lakes, NJ, USA) was used for flow cytometry.

8. Tumorigenesis Assays

Gastric cancer sphere cells presenting LINGO2 by flow cytometry were washed with serum-free HBSS (Gibco BRL, Grand Island, NY, USA) and suspended in serum-free RPMI (Gibco BRL) and Matrigel (BD Biosciences PharMingen) (1:1 volume).[26] Cells were subcutaneously injected into both flanks of either BALB/c nude mouse (OrientBio, Seongnam, Korea) or NOD/SCID mice (Charles River Laboratories, Yokohama, Japan). All animal experiments were approved by the Committee for the Care and Use of Laboratory Animals of Yonsei University College of Medicine. Tumor size was calculated as V (mm^3) = (A^2 x B)/2, with A the perpendicular diameter and B the largest dimension. After 14 to 16 weeks, mice were sacrificed and tumor tissues fixed in 4% paraformaldehyde. For histological evaluation, tissue samples were embedded in paraffin and stained with H & E.

9. CD34 & pVEGFR2 Immunohistochemistry

The mouse cancer tissue slides were prepared from the mouse xenograft model injected with cells sorted by LINGO2 expression and deparaffinized in xylene and rehydrated in a graded ethanol series. Endogenous peroxidase activity was quenched by immersing the slide in 0.3% hydrogen peroxide in methanol at room temperature for 20 minutes. Antigen retrieval was performed in citrate buffer (0.01M, pH 6.0) by microwave for 5 minutes. The slides were blocked by soaking in 10 % normal donkey serum in PBS buffer for 1 hour to remove non-specific background. For the analysis of

angiogenesis, the slides were incubated overnight with rat monoclonal anti-CD34 (1:50, Abcam plc) and rabbit polyclonal anti-phospho-VEGFR2 (vascular endothelial growth factor receptor2) (1:50, Cell signaling technology, Inc.) at 4℃. For the evaluation of CD34 expression, the slides were incubated with anti-rat IgG-HRP (1:200, Santa Cruz Biotechnology) as the secondary antibody for 1 hour at room temperature. Analysis of pVEGFR2 expression was performed using Envision kit (DakoCytomation California, Inc.) in accordance with the manufacturer's instruction. Finally, the slides were incubated with 3,3'-diaminobenzidine (DakoCytomation California, Inc.) and counterstained with modified harris hematoxylin (Sigma-Aldrich).

10. LINGO2 Knockdown

To verify the inhibitory effects of LINGO2 expression, LINGO2 shRNA plasmid or mock shRNA plasmid (Sure Silencing shRNA plasmids) from Qiagen was transfected into SNU484 cells to establish a LINGO2 knockdown cell line. ShRNA sequence against LINGO2 was AGA CTT GAG TGA CAA CAT CAT and mock shRNA was GGA ATC TCA TTC GAT GCA TAC. Selection was by puromycin (2 μg/mL). Transfection of LINGO2 shRNA plasmid or mock shRNA plasmid was with Lipofectamine 2000 (Invitrogen). After 24 hours transfection, media were replaced by media with 2 μg/mL of puromycin for 3 days. Surviving cells were cultured in 100 mm plate at 20-30 cells per plate and single colonies selected using a cloning cylinder (Sigma-Aldrich). Western blots and flow cytometry were performed to determine the effects of LINGO2 knockdown.

11. Cell Proliferation Assays

Cells at 1.1 x 10^4 were seeded into 24-well plates, detached using 0.25% trypsin-EDTA every two days for 8 days and counted using a hematocytometer.

12. Wound-healing Assays

Cells were grown to nearly 100% confluence and linear wounds were created in the confluent monolayer using pipette tips. Wound healing ability was observed over time under a microscope.

13. Migration and Invasion Assays

Migration and invasion of cancer cells was determined in Transwells (Corning Inc., Tewksbury, MA, USA). For invasion assays, matrigel (1:4, BD Biosciences) was added to Transwell membrane filter inserts with 8 μm pores (Costar, Cambridge, MA, USA) and incubated for 1 hour in a 5% CO_2 tissue incubator. Cells (1 x 10^4 cells/well) were placed in the upper chamber in culture medium and the bottom chamber was filled with culture medium from NIH3T3 fibroblasts followed by incubation for 24 hours for migration assays or 72 hours for invasion assays. Degree of migration/invasion was determined by counting cells that migrated through the membrane at three randomly chosen visual fields per filter.

14. Vimentin Immunofluorescence Staining

Cultured cells were fixed in methanol and immunostained with mouse monoclonal anti-vimentin (1:100, Santa Cruz Biotechnology) overnight at 4℃. Stained cells were incubated with goat anti-mouse Cy3-conjugated secondary antibody (Jackson ImmunoResearch Inc., West Grove, PA, USA) for 30 minutes. Nuclei were labeled with 4′,6-diamidino-2-phenylindole (DAPI). Stained cells were analyzed on an Olympus BX51 microscope and images captured using an Olympus DP71 camera (Olympus America Inc., Center Valley, PA, USA)

15. Zymography

Zymography was used to evaluate the gelatinase activity of MMP2 and MMP9 in both mock and LINGO2 knockdown cells. In 100-mm dishes containing serum-free RPMI medium (Invitrogen), 5 x 10^5 cells were cultured for 48 hours. Supernatant was retrieved by centrifugation and concentrated using Amicon ultrafilter devices with a 3 kDa molecular-mass cutoff (Millipore, Tullagreen, Ireland). Concentrated solution (20 μl) was mixed with sample buffer at 1:1 and loaded onto 10% zymogram gels (Bio-rad, Hercules, CA, USA) followed by electrophoresis at 100 V for 90 minutes. Gels were immersed in buffer containing 2.5% Triton-X 100 and renatured for 30 minutes at room temperature. Buffer was replaced by development solution (Bio-Rad) and gels were incubated overnight at 37℃

then stained with Coomassie blue (Bio-Rad) and destained to show clear bands indicating gelatinase activity of MMP2 and MMP9. Gels were dried for scanning.

16. Flow Cytometry for CD44

Cells were dissociated with Accutase (Sigma Aldrich) and 1 x 10^6 cells were labeled with anti-CD44-fluorescein isothiocyanate (BD Biosciences PharMingen). Labeled cells were detected using a BD LSRII flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

17. Statistical Analysis

Continuous data are expressed as means ± SD or median when appropriate. Categorical data are expressed as the number or proportion of subjects with a specified condition or clinical variable. Significance of differences between categorical data were evaluated using a chi-square test. Relationships between two quantitative, continuous variables were investigated by bivariate correlation analysis. Mean overall survival was estimated by Kaplan-Meier analysis with 95% confidence intervals (95% CIs). All analysis was performed using SPSS, ver. 18.0 (IBM SPSS statistics, Chicago, Il, USA) and $p < 0.05$ was considered significant.

III. RESULTS

1. Tumor Sphere Formation by Human Gastric Cancer Cells

Formation of tumor spheres was studied in 9 human gastric cancer cell lines. Among them, N87 and SNU484 gastric cancer cells formed tumor spheres (Fig. 1).

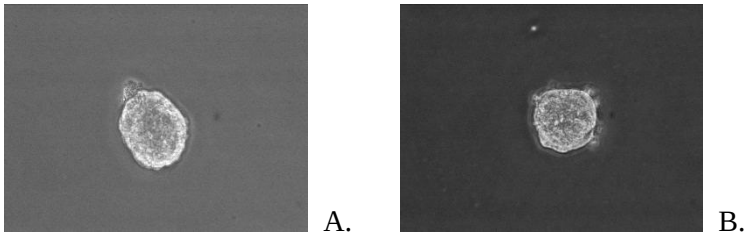


Figure 1. Formation of tumor sphere in gastric cancer cell lines (A,N87; B, SNU484)

We confirmed expression of stemness-related molecules Jagged2, Notch3, Hes1, Ihh, Smo, Gli1, Fzd7, β-catenin, Nanog, Oct4 and PTEN in tumor spheres from gastric cancer cell lines. We also evaluated EMT-related markers in N87 and SNU484 gastric cancer sphere cells by western blot. Mesenchymal markers twist, N-cadherin and α-smooth muscle actin (α-SMA) were expressed in gastric cancer sphere cells. In contrast, the epithelial marker occludin was poorly expressed in sphere cells (Fig. 2).

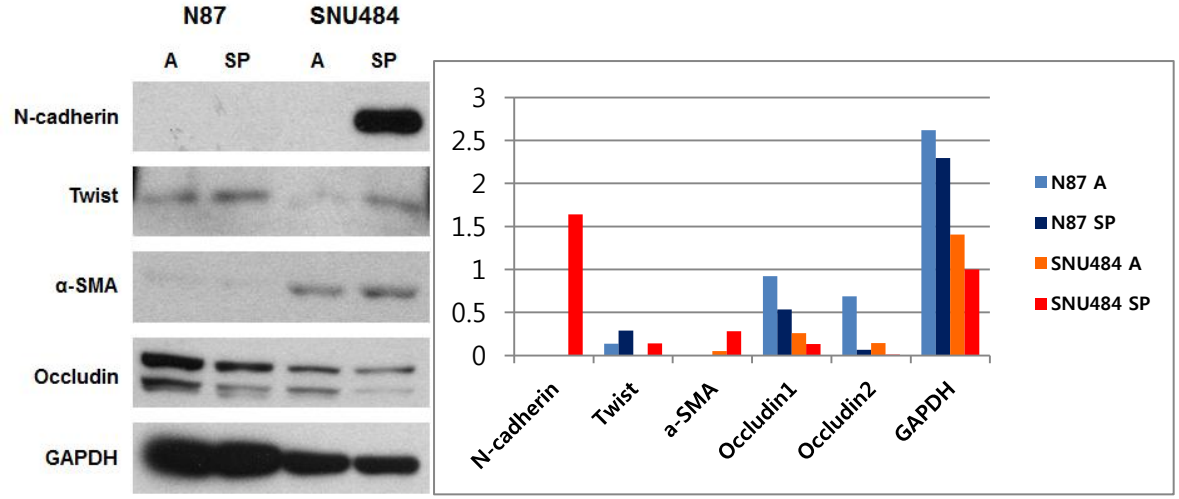


Figure 2. Protein expression of EMT markers by western blot and densitometry in gastric cancer sphere cells. Mesenchymal marker, N-cadherin, Twist, and α-SMA were highly expressed and epithelial marker occludin was poorly expressed in gastric cancer sphere cells. (A, attached control cells; SP, sphere cells) (Blue bar, N87 cell line; Red bar, SNU484 cell line)

2. Validation and Expression of LINGO2 in Gastric Cancer

We performed DNA microarrays on gastric cancer sphere cells to find novel gastric CSC-related markers. Membranous or secretory proteins were selected as candidates. The membrane protein

LINGO2 was upregulated with 1.31 in N87 cells and 2.88-fold changes in SNU484 cells. We compared LINGO2 expression in gastric cancer sphere cells with attached control cells. Both LINGO2 mRNA and protein were more highly expressed in gastric cancer sphere cells than attached cells (Fig. 3).

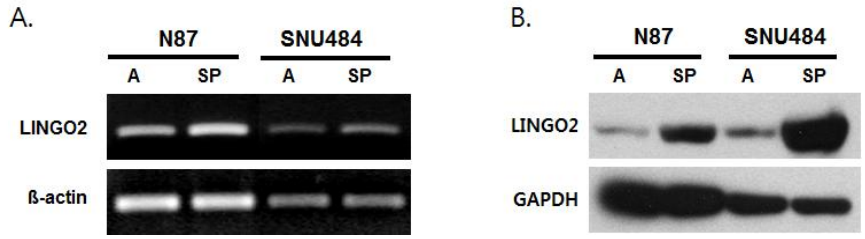


Figure 3. Increased LINGO2 expression in gastric cancer sphere cells. A, RT-PCR. B, western blot. (A, attached control cells; SP, sphere cells) Both mRNA (A) and protein (B) of LINGO2 were highly expressed in gastric cancer sphere cells.

We confirmed expression of LINGO2 in the gastric cancer cell lines AGS, N87, SNU1, SNU5, SNU16, SNU484, SNU601, SNU638 and SNU668 using RT-PCR (Fig. 4).

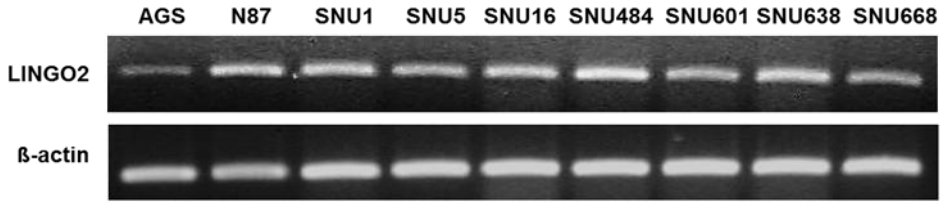


Figure 4. Expressions of LINGO2 mRNA in human gastric cancer cell lines by RT-PCR. LINGO2 was expressed in the nine gastric cancer cell lines

We further evaluated LINGO2 expression in 113 samples in human gastric cancer TMAs by immunohistochemistry (IHC) (Fig. 5). We divided into two groups by LINGO2 expression. For statistical analysis, LINGO2 IHC staining scores of 0 or 1 were considered negative. LINGO2 was highly expressed in 31 samples of the LINGO2-positive group.

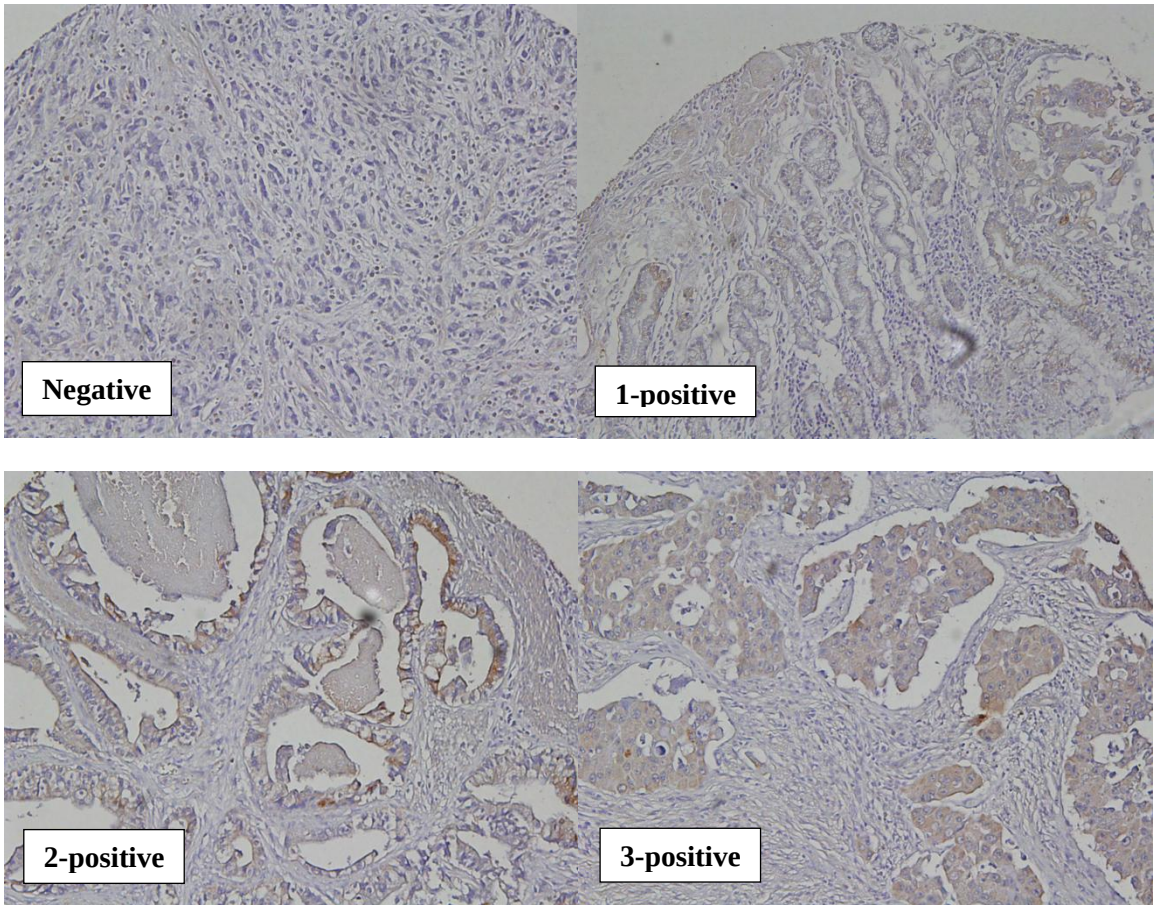


Figure 5. LINGO2 expression in human gastric cancer tissues by immunohistochemistry.

TMA samples of 113 gastric cancer tissues were stained with anti-LINGO2 antibody. LINGO2 staining was scored as percentage of LINGO2-positive tumor cells: no expression, negative; <20%, 1-positive; 20-50%, 2-positive; and >50%, 3-positive. Numbers of patients in each score were 58, 24, 15 and 16, respectively. 2 or 3- positive IHC staining scores of LINGO2 considered positive group. Advanced lymph node invasion correlated positively with LINGO2-positive expression.

Clinical characteristics of LINGO2 IHC-positive and negative samples are in Table 2.

Table 2. Clinical characteristics according to LINGO2 expression by IHC.

IHC	LINGO2-negative	LINGO2-positive	p-value
Number of cases	82 (72.6%)	31 (27.4%)	
Age (mean)	58.3±10.9	56.8±11.3	
Sex (M/F)	54 / 28	19 / 12	0.665
T (T1/T2/T3/T4)	29/21/29/3	8/4/17/2	0.190

N (N0/N1/N2/N3)	39/19/19/5	9/7/9/6	0.102
M (M0/M1)	79/3	27/4	0.088
Stage (I/II/III/IV)	46/16/17/3	12/4/11/4	0.073

LINGO2 expression correlated positively with advanced nodal (r = +0.220, p = 0.019), and TNM stage (r = +0.211, p = 0.025). Higher LINGO2 expression in gastric cancer tissues was associated with lymph node invasion and advanced staging. However, no significant differences were seen in survival days between the positive and negative LINGO2-staining groups. Mean years of survival for the LINGO2 IHC-positive group were 8.21 ± 1.13 years (95% CI, 6.00–10.42 years), compared to 8.96 ± 0.68 years (95% CI, 7.63–10.29 years) for the LINGO2-negative group in Figure 6 (p = 0.386).

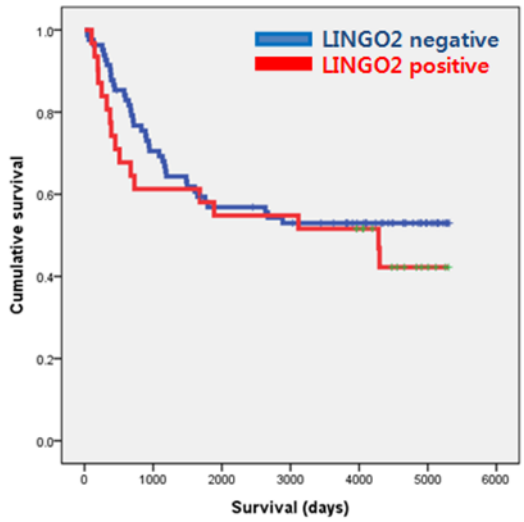


Figure 6. Mean survival days according to LINGO2 expression (blue line, LINGO2-negative group; Red line, LINGO2-positive group). Mean survival years for the LINGO2 IHC-positive group were 8.21 ± 1.13 years (95% CI, 6.00–10.42 years), compared to 8.96 ± 0.68 years (95% CI, 7.63–10.29 years) for the LINGO2-negative group (p = 0.386).

3. Enhanced Tumorigenesis and Overexpression of Stemness-related Molecules in LINGO2-high Gastric Cancer Sphere Cells

LINGO2 is a cell surface marker so gastric cancer sphere cells derived from the SNU484 cell line were sorted by LINGO2 expression into LINGO2-high and LINGO2-low cells. LINGO2-high cells actively migrated in migration assays (Fig. 7).

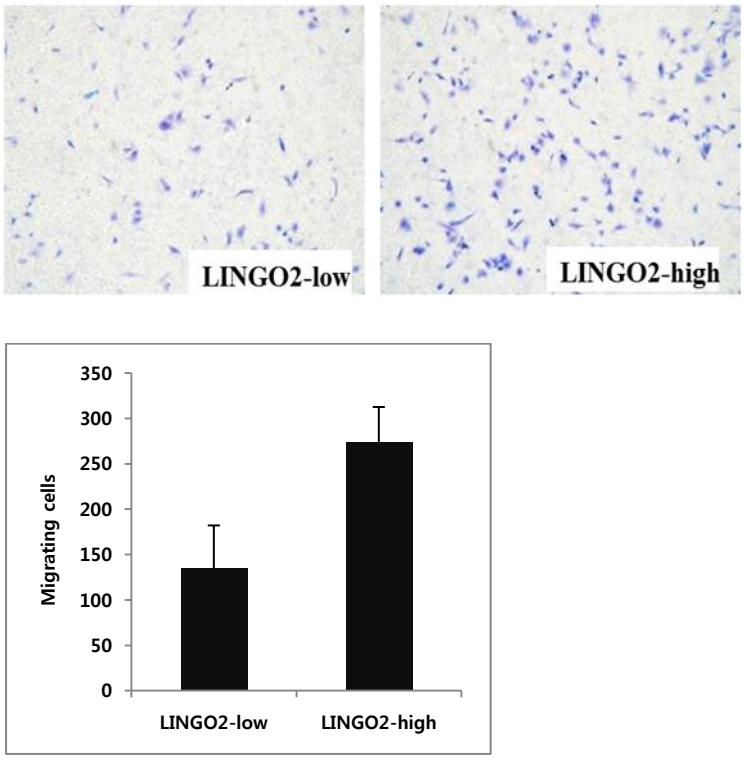


Figure 7. Migration assay of LINGO2-high cells derived from SNU484 gastric cancer sphere cells. LINGO2-high cells were higher migrated than LINGO2-low cells.

In addition, LINGO2-high gastric cancer sphere cells overexpressed stemness-related molecules. Oct4, PTEN, Gli1 and Hey1 expression were analyzed by western blots and all were much higher expressed in LINGO2-high than LINGO2-low gastric cancer sphere cells (Fig. 8).

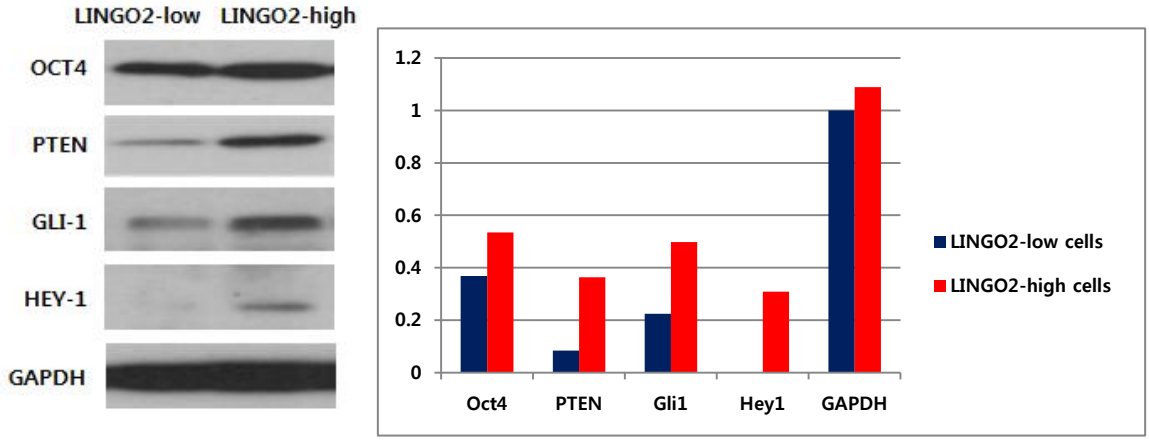


Figure 8. Overexpression of stemness-related molecules in LINGO2-high sphere cells by western blot and densitometry. (Blue bar, LINGO2-low cells; Red bar, LINGO2-high cells) Stemness-related molecules Oct4, PTEN, Gli1 and Hey1were much higher expressed in LINGO2-high sphere cells than LINGO2-low sphere cells.

Tumorigenic properties of LINGO2-high cells were demonstrated using xenograft mice and indicated the ability of isolated tumor cells with stemness to generate tumors. Xenograft tumor sizes for LINGO2-high sphere cells were larger than for LINGO2-low sphere cells (Fig. 9).

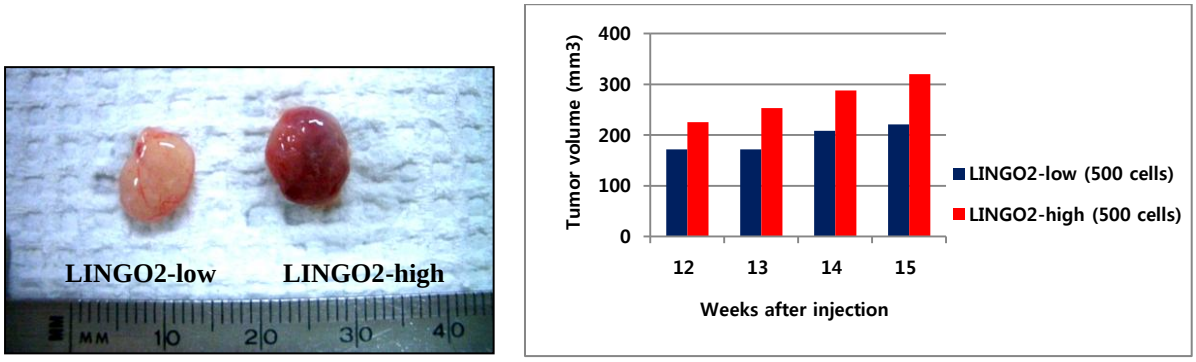


Figure 9. Enhanced tumorigenesis in xenograft BALB/c nude mouse with LINGO2-high gastric cancer sphere cells. Tumor size of LINGO2-high sphere cells were larger than LINGO2-low cells.

Xenograft tumor from LINGO2-high cells showed suggestive of vessel formation. Angiogenesis markers CD34 and p-VEGFR2 were also highly expressed in LINGO2-high sphere cells induced xenograft tumors of NOD/SCID mice by IHC (Fig 10).

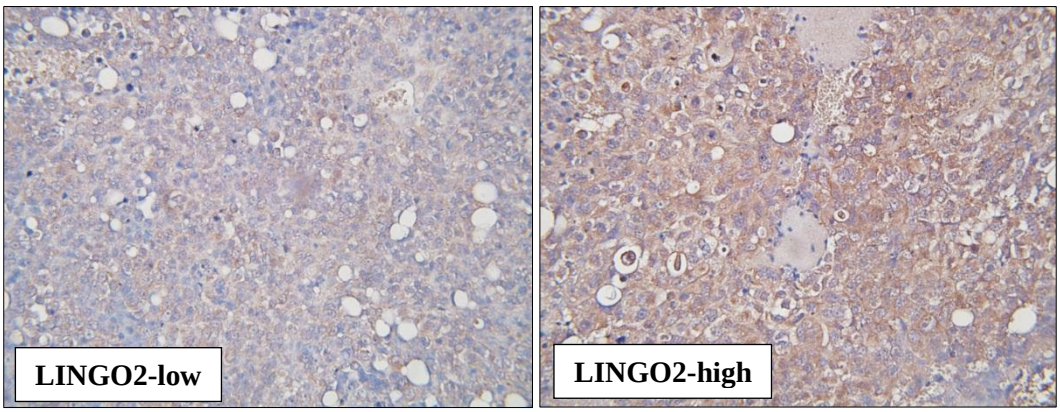


Figure 10. Angiogenesis marker p-VEGF2R expression in LINGO2-high sphere cells induced xenograft tumors of NOD/SCID mice by IHC. p-VEGF2R were higher expressed in LINGO2-high sphere cells than LINGO2-low cells.

4. Decreased Migration and Invasion of LINGO2 knockdown cells

LINGO2 knockdown cells, established using shRNA, downregulated LINGO2 by western blot (Fig. 11).

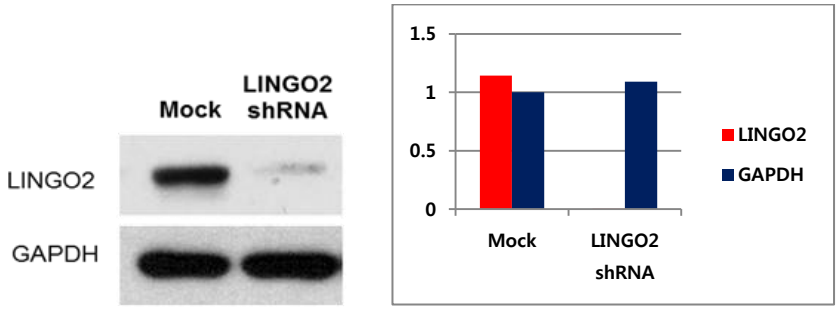


Figure 11. LINGO2 knockdown was confirmed by western blot showing downregulation of LINGO2 in LINGO2 shRNA cells.

In cell proliferation assays, LINGO2 knockdown cells had lower proliferative ability than mock cells (Fig. 12). Wound healing assays evaluated proliferation and migration. Delayed wound healing was seen for LINGO2 shRNA cells (Fig. 13). LINGO2 shRNA cells also had low migration and invasion ability (Fig. 14, 15). Thus, inhibition of LINGO2 by shRNA reduced cellular migration and invasion.

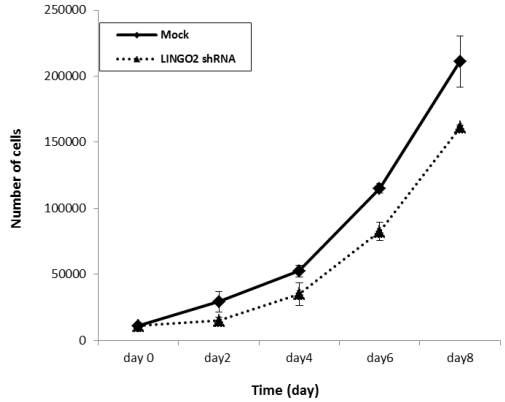


Figure 12. Cell proliferation assays of LINGO2 knockdown cells. (Solid line, mock cells; dotted line, LINGO2 shRNA cells) LINGO2 shRNA knockdown cells decreased cellular proliferation than mock cells.

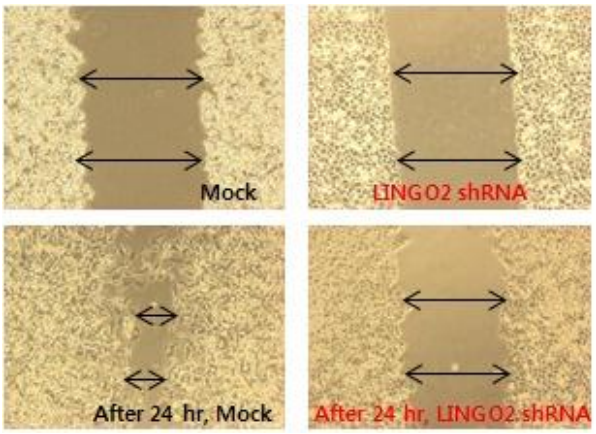
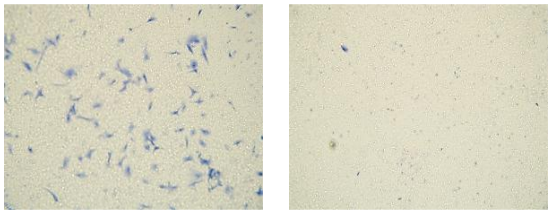


Figure 13. Wound-healing assays with LINGO2 knockdown cells. Wound healing assay showed delayed healing process in LINGO2 knockdown cells.



Mock LINGO2 shRNA

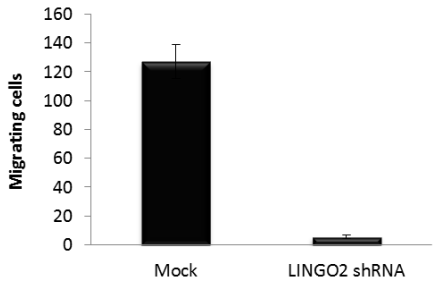


Figure 14. Migration assays with LINGO2 knockdown cells. Migration assay showed less migratory ability in LINGO2 knockdown cells.

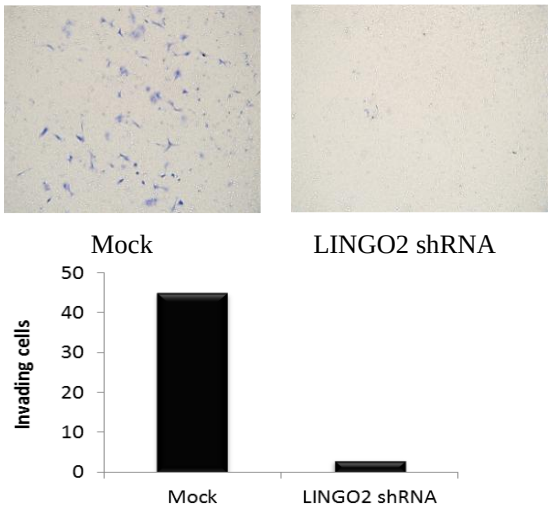


Figure 15. Invasion assays with LINGO2 knockdown cells. Invasion assay showed less invasive ability in LINGO2 knockdown cells

5. Knockdown of LINGO2 Inhibits Mesenchymal Marker Expression in EMT and AKT/ERK Signal Pathways

To examine LINGO2 effects on EMT, mesenchymal markers expression was determined by immunofluoroscence staining or western blots of LINGO2 shRNA cells. N-cadherin expression was inhibited in LINGO2 shRNA cells compared to mock cells. LINGO2 knockdown increases the expression of epithelial cell marker, occludin in LINGO2 shRNA cells (Fig. 16).

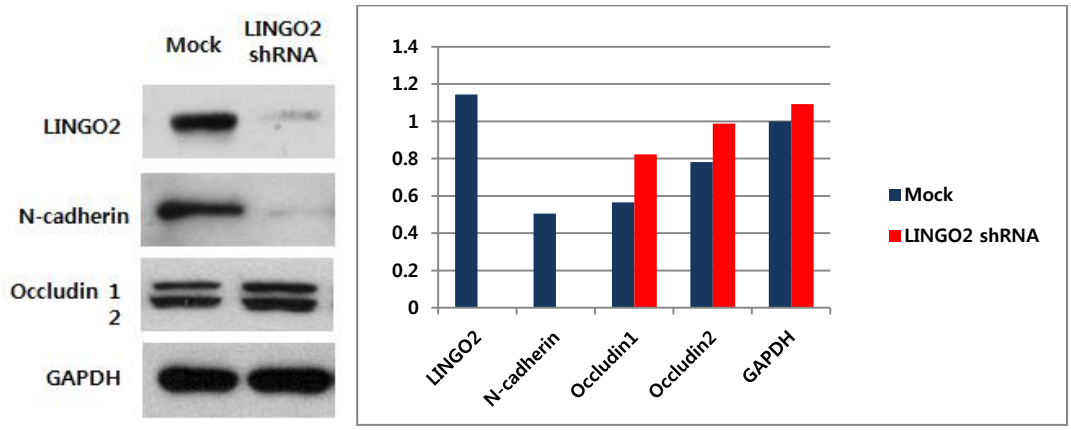
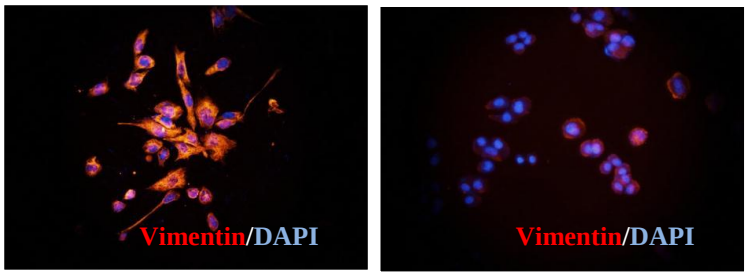


Figure 16. Inhibition of the mesenchymal marker N-cadherin in LINGO2 knockdown cells by western blot and densitometry. (Blue bar, mock cells; Red bar, LINGO2 shRNA cells)

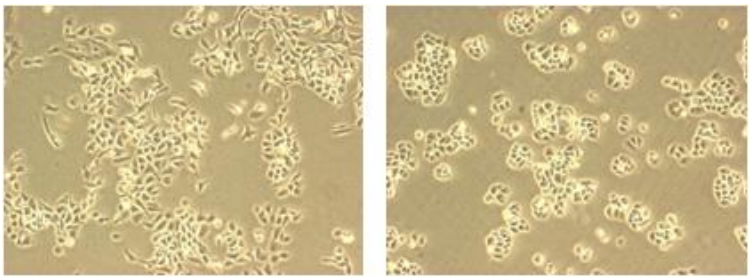
Expression of vimentin was also decreased in LINGO2 shRNA cells by immunofluoroscence staining (Fig. 17).



Mock LINGO2 shRNA

Figure 17. Expression of vimentin was reduced in LINGO2 knockdown cells by immunofluorescence staining. LINGO2 knockdown cells decreased the expression of EMT marker, vimentin. And cellular phenotype was changed from mesenchymal cells appearance in mock cells to epithelial cells.

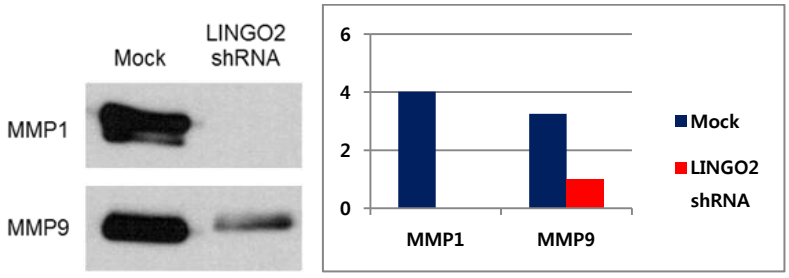
In LINGO2 shRNA cells, the mesenchymal-cell phenotype was reversed and cells showed the characteristics of epithelial cells. LINGO2 knockdown changed mesenchymal cellular morphology like elongated fibroblast to cobblestone-like epithelial cells (Fig. 18).



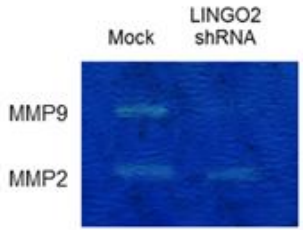
Mock LINGO2 shRNA

Figure 18. LINGO2 knockdown changes cellular morphology from spindle-like mesenchymal cells to cobblestone-like epithelial type cells.

LINGO2 knockdown cells had decreased expression of MMP1 and MMP9 by western blots. We also confirmed reduced MMP9 activity by zymography of LINGO2-shRNA cells (Fig. 19).



A.



B.

Figure 19. Expressions of MMP1 and MMP9 in LINGO2 knockdown cells by western blot (A) and zymography (B). LINGO2 shRNA cells inhibited the protein expressions of MMP1 and MMP9 by western blot. Zymography decreased the activity of MMP9.

We checked the alterations of signal pathway in LINGO2 knockdown cells. LINGO2 shRNA cells inhibited expression of pAKT, pMEK and pERK. We found that knockdown of LINGO2 decreased AKT/ERK phosphorylation (Fig. 20).

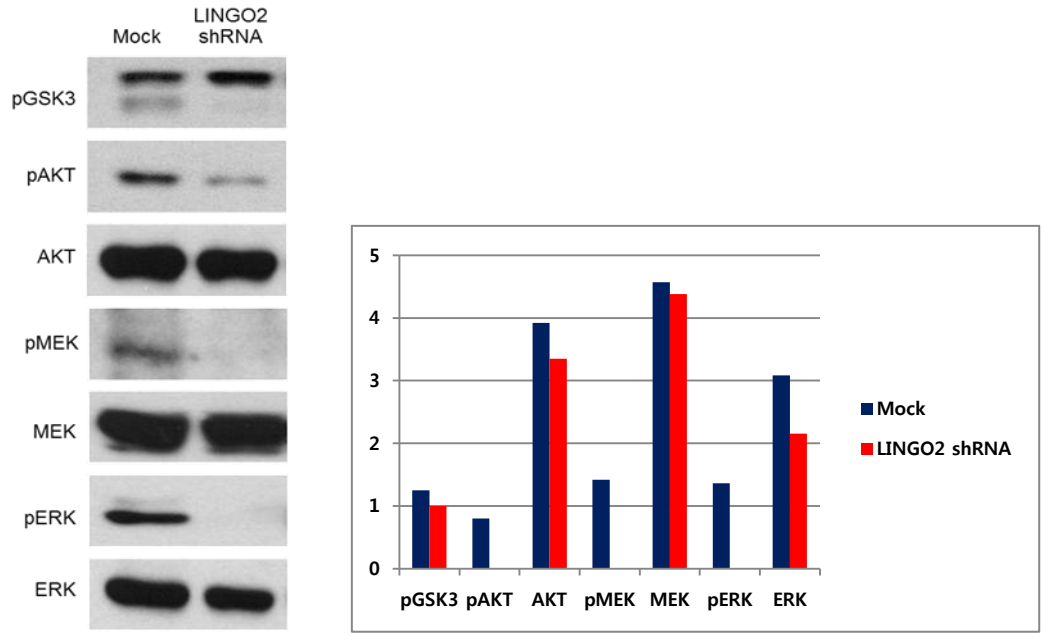
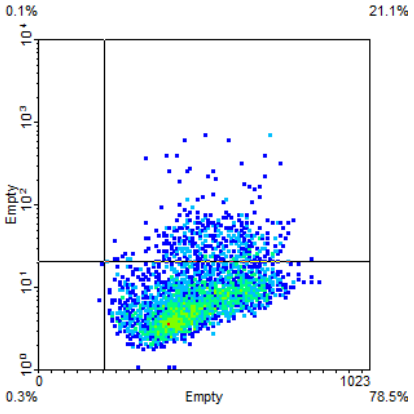


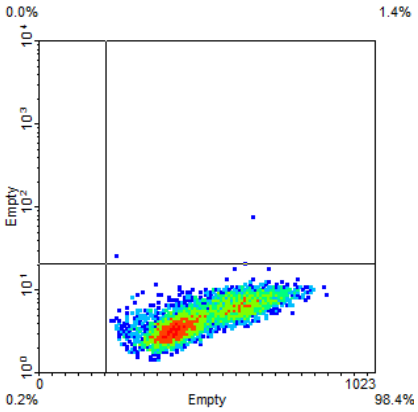
Figure 20. Expression of pAKT, pMEK and pERK in LINGO2 shRNA cells by western blot and densitometry. LINGO2 knockdown cells inhibited expression of pAKT, pMEK and pERK

6. Flow Cytometry for CD44 in LINGO2 knockdown cells

CD44 expression was evaluated in LINGO2-shRNA cells by flow cytometry. CD44 was expressed in 19.7 ± 0.85 % of mock cells and 0.8 ± 0.14 % of LINGO2-shRNA cells (Fig. 21). CD44 expression was downregulated in LINGO2 knockdown cells.



A.



B.

Figure 21. CD44 expression s of LINGO2 knockdown cells by flow cytometry. LINGO2 shRNA cells inhibited CD44 expression. (A, Mock cells; B, LINGO2 shRNA cells)

IV. DISCUSSION

The cancer stem cell theory, which was demonstrated by Reya *et al.* in 2001,[27] proposes that cancers are derived from a stem cell compartment in a multistep process involving the accumulation of mutations in a variety of tumor suppressors and oncogenes.[19, 28] The crucial debate about the origin of CSCs is if CSCs are derived from stem cells that acquire stemness or mimic stem cells because of microenvironments affected by the stroma, indicating epigenetic alterations.[29] CSCs have a specific surface marker. CD44, the most widely known CSC marker, is expressed in various cancers.[21,30] Thus, cells sorted for CSC surface expression can be used to analyze the characteristics of CSCs and determine relationship to cancer progression.

In this study, we discovered a novel gastric CSC-related marker, LINGO2, and analyzed its function in gastric cancer. LINGO2 was selected from DNA microarrays derived from gastric cancer sphere cells. LINGO2, a human paralog of LINGO1 mainly expressed in neuronal tissues, is little known. In our experiments, LINGO2 is almost not expressed in the most human normal organs except stomach and partial portion of colon by IHC (data not shown). In human gastric cancer tissues, LINGO2 were strongly expressed about 27% of patients in our data.

Furthermore, LINGO2-high sphere cells expressed stemness-related molecules. And the mesenchymal markers N-cadherin, vimentin, MMP9 were downregulated in LINGO2 shRNA-knockdown cells. We suggest that cancer stemness-related marker LINGO2 is closely associated with the EMT process in gastric cancer.

Most gastric cancers are related to *Helicobacter pylori* infections causing chronic inflammation. Yin *et al.* showed that *H. pylori* induced an EMT-like process in gastric epithelial cell lines *in vitro*.[31] In a study of a transgenic mouse model of gastric carcinogenesis, MMP7 was upregulated by *H. pylori*. In addition, *H. pylori* induced EMT markers via CagA with upregulation of mesenchymal markers ZEB1, vimentin, Snail1, Twist, BMP1 and MMP9 and downregulation of cytokeratins as epithelial markers in Baud *et al*.[32,33] *H. pylori* produces tumor necrosis factor-α-inducing proteins related to enhanced migration and elongation of human gastric cancer cell lines. They also increase expression of vimentin, a mesenchymal marker, through activation of the MEK-ERK signal pathway.[34]

Although *H. pylori* infection status was not identified in the clinical data of this study, EMT pathway through the activation of MEK-ERK signaling by LINGO2 activation was suggested from the results of functional study for LINGO2. The cellular phenotype from mesenchymal to epithelial cells reversed in LINGO2 shRNA cells. In addition, LINGO2-knockdown cells had inhibited MEK/ERK phosphorylation. Clinically, LINGO2 expression correlated positively with advanced nodal stage in the human stomach. Nodal stage in gastric cancer is known to be a predictive factor of recurrence. However, LINGO2 expression is not affected in the overall survivals of gastric cancer patients. It could be suggested that many early gastric cancer patients were enrolled in the TMA samples. Thus, considerable number of patients survived until now.

Recent studies revealed that CD44v6, a special variant of CD44, is related to angiogenesis and VEGF2 signaling.[35] Reduced CD44 expression in LINGO2 shRNA knockdown cells might be either a CSC-related phenomenon or angiogenesis. Therefore, LINGO2 is important in gastric cancer invasion and metastasis associated with EMT pathway.

V. CONCLUSION

LINGO2 is a novel, gastric, CSC-related marker with potential clinical implications for gastric cancer treatment. LINGO2 enhanced cellular migration and invasion in gastric cancer cells closely associated with EMT pathway. Clinically, LINGO2 expression correlated positively with advanced lymph node invasion, a known predictive factor of recurrence in human gastric cancer. Thus, we focused LINGO2 on cancer invasion and metastasis. Inhibition of LINGO2 could be useful in targeted therapies for gastric cancer.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
2. Peng Z, Wang CX, Fang EH, Wang GB, Tong Q. Role of epithelial-mesenchymal transition in gastric cancer initiation and progression. World J Gastroenterol 2014;20:5403-10.
3. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, et al. Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer research 2006;66:9339-44.
4. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nature Reviews. Cancer 2008;8:755-68.
5. Yang L, Ping Y, Yu X, Qian F, Guo Z, Qian C, et al. Gastric cancer stem-like cells possess higher capability of invasion and metastasis in association with a mesenchymal transition phenotype. Cancer letters 2011;310:46-52.
6. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 2005;5:275-84.
7. Blagosklonny MV. Target for cancer therapy: proliferating cells or stem cells. Leukemia 2006;20:385-91.
8. Kruger GM, Morrison SJ. Brain repair by endogenous progenitors. Cell 2002;110:399-402.
9. Lou H, Dean M. Targeted therapy for cancer stem cells: the patched pathway and ABC transporters. Oncogene 2007;26:1357-60.
10. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote

radioresistance by preferential activation of the DNA damage response. Nature 2006;444:756-60.

11. Floor S, van Staveren WC, Larsimont D, Dumont JE, Maenhaut C. Cancer cells in epithelial-to-mesenchymal transition and tumor-propagating-cancer stem cells: distinct, overlapping or same populations. Oncogene 2011;30:4609-21.
12. Mani SA, Guo W, Liao M, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008;133:704-15.
13. Morel A, LiÃ vre M, Thomas C, Hinkal G, Ansieau S, Puisieux A. Generation of breast cancer stem cells through epithelial-mesenchymal transition. PLoS ONE 2008;3:e2888-e.
14. Santisteban M, Reiman JM, Asiedu MK, Behrens MD, Nassar A, Kalli KR, et al. Immune-induced epithelial to mesenchymal transition in vivo generates breast cancer stem cells. Cancer research 2009;69:2887-95.
15. ED H. Organization and fine structure of epithelium and mesenchyme in the developing chick embryo. Baltimore: Williams and Wilkins; 1968. p.31–55.
16. Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer 2002;2:563-72.
17. Nguyen DX, Bos PD, Massague J. Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer 2009;9:274-84.
18. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell 2011;147:275-92.
19. Baum B, Settleman J, Quinlan MP. Transitions between epithelial and mesenchymal states in development and disease. Semin Cell Dev Biol 2008;19:294-308.
20. Cairns J. Mutation selection and the natural history of cancer. Nature 1975;255:197-200.
21. Takaishi S, Okumura T, Tu S, Wang SS, Shibata W, Vigneshwaran R, et al. Identification of gastric cancer stem cells using the cell surface marker CD44. Stem cells 2009;27:1006-20.
22. Ryu HS, Park do J, Kim HH, Kim WH, Lee HS. Combination of epithelial-mesenchymal transition and cancer stem cell-like phenotypes has independent prognostic value in gastric cancer. Hum Pathol 2012;43:520-8.
23. Mi S, Lee X, Shao Z, Thill G, Ji B, Relton J, et al. LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. Nat Neurosci 2004;7:221-8.
24. Vilarino-Guell C, Wider C, Ross OA, Jasinska-Myga B, Kachergus J, Cobb SA, et al. LINGO1 and LINGO2 variants are associated with essential tremor and Parkinson disease. Neurogenetics 2010;11:401-8.
25. Delay C, Tremblay C, Brochu E, Paris-Robidas S, Emond V, Rajput AH, et al. Increased LINGO1 in the cerebellum of essential tremor patients. Mov Disord 2014.
26. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic

cancer stem cells. Cancer Res 2007;67:1030-7.

27. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001;414:105-11.
28. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. Science 1977;197:461-3.
29. Tu SM. Cancer: a "stem-cell" disease? Cancer Cell Int 2013;13:40.
30. Hong SP, Wen J, Bang S, Park S, Song SY. CD44-positive cells are responsible for gemcitabine resistance in pancreatic cancer cells. Int J Cancer 2009;125:2323-31.
31. Yin Y, Grabowska AM, Clarke PA, Whelband E, Robinson K, Argent RH, et al. Helicobacter pylori potentiates epithelial:mesenchymal transition in gastric cancer: links to soluble HB-EGF, gastrin and matrix metalloproteinase-7. Gut 2010;59:1037-45.
32. Bagnoli F, Buti L, Tompkins L, Covacci A, Amieva MR. Helicobacter pylori CagA induces a transition from polarized to invasive phenotypes in MDCK cells. Proc Natl Acad Sci U S A 2005;102:16339-44.
33. Bessede E, Staedel C, Acuna Amador LA, Nguyen PH, Chambonnier L, Hatakeyama M, et al. Helicobacter pylori generates cells with cancer stem cell properties via epithelial-mesenchymal transition-like changes. Oncogene 2014;33:4123-31.
34. Watanabe T, Takahashi A, Suzuki K, Kurusu-Kanno M, Yamaguchi K, Fujiki H, et al. Epithelial-mesenchymal transition in human gastric cancer cell lines induced by TNF-alpha-inducing protein of Helicobacter pylori. Int J Cancer 2014;134:2373-82.
35. Tremmel M, Matzke A, Albrecht I, Laib AM, Olaku V, Ballmer-Hofer K, et al. A CD44v6 peptide reveals a role of CD44 in VEGFR-2 signaling and angiogenesis. Blood 2009;114:5236-44.

<ABSTRACT (IN KOREAN)>

새로운 위암 줄기세포 관련 표지자인 LINGO2에 의한 상피 중간엽 전이와 연관된 위암세포의 이동 및 침윤 증가

연세대학교 대학원 의학과

박세미

<지도교수 송시영>

연구배경: 암 줄기세포는 정상줄기세포와 유사하게 다양하게 분화할 수 있는 자기 복제 능력을 가지고 있는 소수의 암세포 군으로 추정되는 세포이다. 이 세포는 또한 항암제나 방사선 치료에 내성을 가지고 있는 세포로써 최근에는 이러한 암 줄기세포가 암 발병 및 다른 장기로의 침윤 및 전이에 관여하여 암의 치료를 어렵게 하여 결과적으로 암의 예후를 좋지 않게 하는 것으로 알려져 왔고 이에 대한 연구가 활발하게 진행되고 있다.

연구목적: 본 연구의 목적은 위암 세포주의 암줄기세포 성질을 가지고 있는 종양 sphere 세포에서 찾아낸 새로운 위암 줄기세포 표지자인 LINGO2의 기능을 알아내는 것이다.

연구방법: 새로운 위암 줄기세포와 연관된 표지자를 찾아내고자 위암 세포주에서 형성한 종양 sphere 세포를 일반 종양세포와 비교하여 디옥시리보 핵산 미세배열 기술(DNA microarray)로 분석하였다. 그 결과, 과발현 된 유전자 중에서 LINGO2 를 찾아내었고 LINGO2 를 역전사 중합효소 연쇄반응, 웨스턴 블랏 및 면역 화학 염색 등을 시행하였다. 또한 LINGO2 의 기능을 확인하고자 유동세포 분석법을 이용하여 LINGO2의 발현여부에 따라 위암 sphere 세포를 분리하여 면역저하 생쥐에 이식하여 종양 발생을 확인하였다. 또한, SNU484 위암 세포주에서 짧은 헤어핀 리보핵산(shRNA)을 이용하여 LINGO2 발현이 억

제된 세포를 만들어 분석하였다.

결과: LINGO2 는 여러 위암 세포주 및 위암 sphere 세포에서 발현이 증가되었다. 사람 위암 조직에서는 약 27퍼센트에서 LINGO2 가 발현되었고 병기가 진행될수록, 임파절 전이가 심해질수록 LINGO2의 발현이 증가하는 양의 상관관계를 보였다. 그러나, LINGO2 의 발현과 전체 위암환자의 생존률 사이에는 유의한 차이를 보이지는 않았다. 또한 유동세포분석법을 이용하여 분리한 LINGO2 고발현 세포는 저발현 세포에 비해 세포이동이 증가하였고, 면역저하 생쥐에서의 종양 형성을 확인하였다. LINGO2 발현이 억제된 knockdown 세포에서는 LINGO2 발현 sphere 세포와 비교시, 세포 이동 및 침윤이 감소하였고, 중간엽세포 표지자인 N-카드헤린 및 기질금속단백질(matrix metalloproteinase) 등의 발현이 억제되었다.

결론: 새로운 위암 줄기세포 관련 표지자인 LINGO2 는 상피 중간엽 전이 과정과 연관되어 위암 세포의 침윤 및 전이가 증가하는 것을 확인할 수 있었다. 이러한 결과로 LINGO2 가 잠재적으로 위암의 새로운 표적 치료 목표가 될 수 있겠다.

핵심되는말 :암줄기세포; 위암; LINGO2; 상피 중간엽 전이; 세포침윤; 세포이동

PUBLICATION LIST

Park S, Chung MJ, Park JY, Chung JB, Bang S, Park SW, Song SY. Phase II Trial of Erlotinib Plus Gemcitabine Chemotherapy in Korean Patients with Advanced Pancreatic Cancer and Prognostic Factors. Gut Liver. 2013;7:611-5.

Park S, Park JY, Chung MJ, Chung JB, Park SW, Han KH, Song SY, Bang S. The efficacy of endoscopic palliation of obstructive jaundice in hepatocellular carcinoma. Yonsei Med J. 2014;55:1267-72.

Park S, Park JY, Bang S, Park SW, Chung JB, Song SY. Radiotherapy prolongs biliary metal stent patency in malignant pancreatobiliary obstructions. Gut Liver. 2013;7:480-5.

Chung JW, Park S, Chung MJ, Park JY, Park SW, Chung JB, Song SY. A novel disposable, transnasalesophagoscope: a pilot trial of feasibility, safety, and tolerance. Endoscopy. 2012;44:206-9.