ANTICANCER RESEARCH *40*: 1335-1344 (2020)
doi:10.21873/anticanres.14075

Silencing Delta-like 1 Expression Induces Migratory Features in Pancreatic Cancer Cells Through Stimulation of Src and p38 Signalling Pathway

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Abstract. *Background/Aim: The prognosis of pancreatic cancer has not improved due to its migratory feature and refractory potential to chemo-resistance with absence of effective diagnosis. Despite continuous efforts, its underlying mechanisms of malignant nature remain ambiguous. The objective of this study was to investigate delta-like 1 (DLL1) as a tumor suppressor in the metastasic ability of human pancreatic cancer cells. Materials and Methods: Cellular expression of DLL1 was demonstrated using the GEO public database and western blot analysis. The biological function of DLL1 was validated by biological behavior analysis. Prognosis to DLL1 expression was demonstrated using analysis of the GEO public database. Results: Analysis using the GEO database and western blotting showed higher DLL1 mRNA and protein expression levels in pancreatic cancer compared to those in normal pancreas. DLL1 was uniquely expressed in seven human pancreatic cancer cell lines compared to human pancreatic duct epithelial H6c7 cells. Ablation of DLL1 expression stimulated migration and invasion by activating Src and p38 phosphorylation, but not viability and chemo-resistance of human pancreatic cancer cells. In addition, expression of DLL1 was correlated with migratory features of pancreatic cancer in vivo. Moreover, high DLL1 expression was associated with a favorable prognosis in pancreatic cancer patients. Conclusion: DLL1 is a potent suppressor of pancreatic cancer metastasis. Understanding correlation between expression and function of DLL1 might contribute to our knowledge of the complicated mechanism of pancreatic cancer metastasis.*

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Key Words: DLL1, metastasis, suppressor, pancreatic cancer, Src, p38.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most dangerous neoplasms. Its diagnosis rate almost equals its lethality rate (1). Poor survival in PDAC is attributed to its complicated malignant features such as ferocious migratory activity, strong resistance to conventional therapies and arduous diagnosis (2, 3). Thus, it is important to acquire a better understanding of the natural molecular mechanism involved in the development and metastasis of PDAC to inhibit or recognise the rapid spread of this cancer.

Notch signalling is an evolutionarily conserved pathway that regulates a variety of biological processes such as cell fate determination, cellular differentiation, proliferation, survival and tumour angiogenesis by ligand-receptor interaction with neighboring cells (4, 5). In mammals, Notch signalling pathway consists of four transmembrane receptors (Notch-1, -2, -3 and -4) and five ligands (Jagged-1, Jagged-2, Delta-like 1, Delta-like 3 and Delta-like 4) (6). Binding of ligand to its receptor induces the cleavage of Notch receptor which mediates its interaction with transcription factor C promoter-binding factor1/recombination signal binding protein J/k (RBP-J) to activate target genes such as Deltex, hairy enhancer of split (HES) and basic helix-loop-helix (bHLH) transcription factors (7, 8).

The function of Notch signalling in PDAC features has been controversial. Although initial studies have proposed the oncogenic role of Notch signalling, administration of γ-secretase inhibitors has limited anti-cancer effects (9, 10). In addition, recent studies have revealed the tumor-suppressive role of Notch signaling in PDAC development (11, 12). In addition, the function of DLL1 in pancreatic cancer is poorly understood comparing that of DLL1 in other cancer types (13, 14).

Considering conflicting actions of Notch signalling and ambiguous function of DLL1 in PDAC nature, functional DLL1 should be examined as an oncogenic or a suppressive factor for PDAC features. In the present study, we provide the first evidence that DLL1 is a potent suppressor of pancreatic cancer metastasis. This finding might contribute

to the understanding that pancreatic cancer metastasis has complicated mechanism.

Materials and Methods

Gene expression analysis. Microarray expression profiles were obtained from Gene Expression Omnibus (GEO) public microarray database at NCBI (https://www.ncbi.nlm.nih.gov/geo/). Based on histological type, normal pancreas and pancreatic cancer samples were assigned according to its annotation in GEO as described previously (15). Correlations between various anti-cancer drugs and *DLL1* expression levels were analyzed using Cancer Cell Line Encyclopedia (CCLE, https://www.broadinstitute.org/ccle/) public database as described previously (16).

Cell culture. AsPC-1, Capan-1, Capan-2, Miapaca-2, Panc-1, and SNU-213 cells were obtained from the Korean Cell Line Bank (Seoul, Korea). CFPAC-1 was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained as described previously (17). H6c7 was obtained from Kerafast (Boston, MA, USA) and grown as described previously (18). Under standard culture conditions, cells continued the typical morphologies of the original cell line throughout the time period required to do experiments in this study.

Reagents. Antibodies against phospho-ERK1/2 (Thr202/Tyr204), ERK1/2, phospho-FAK (Tyr576/577 and Tye397), FAK, phospho-Src (Tyr416), Src, phospho-p38 (Thr180/Tyr182), p38, DLL1, Notch1 and GAPDH were obtained from Cell Signaling Technology (Beverly, MA, USA). Antibody against phosphor-Tyrosine was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Inhibitor against Src or p38 MAP kinase was obtained from Sigma-Aldrich, in individuals (St. Louis, MO, USA). Different human pancreatic cancer and normal pancreas tissue lysates were purchased from Abcam Cambridge, UK) and Prosci Inc. (Poway, CA, USA).

Transfection of siRNA. Transfection of si-DLL1 was performed using Effectene reagent (Qiagen, Hilden, Germany), as described previously (19). Oligonucleotides specific for DLL1 (sc-94445 and 1042344) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and Bioneer (Daejeon, Korea), in individuals. The oligonucleotide for control siRNA (sc-37007) was obtained from Santa Cruz Biotechnology.

Measurement of cell viability. Cell viability was determined using the WST-1 reagent (Boehringer Mannheim, Mannheim, Germany), as described previously (20). Absorbance at 450 nm was measured using a microplate reader (Bio-Rad, Richmond, CA, USA)

Migration assay. Cell migration assay was performed using 8.0 μm pore size Trans-well permeable supports (Corning Costar, Lowell, MA, USA) as described previously (21). Briefly, AsPC-1, Capan-2, Miapaca-2, Panc-1 and SNU-213 cells (5×10^4/well) were seeded into six-well cell culture plates (Nunc, Roskilde, Denmark). After 18 h, cells were subjected to si-DLL1 transfections and incubated at 37˚C for an additional 48 h. After 12 h starvation with serum-free medium, the migration assay was performed at 37˚C for 6 h, after which cells in the lower surface of the filter were fixed and stained with 1% crystal violet solution. Absorbance of eluted dye was measured at a wavelength of 560 nm using an enzyme-linked immunosorbent assay reader (Bio-Rad, Richmond, CA, USA).

Cell invasion assay. Cell invasion assay was performed using growth factor-reduced Matrigel (BD Biosciences, San Diego, CA, USA) coated on 24-Transwell permeable supports as described previously (22). Cells were layered in the upper chamber containing RPMI without serum for 20 h, and cells that migrated to the back side of the filter after 24 h were stained. The eluted dye was measured at 560 nm in an ELISA reader.

Western blot analysis. To determine the intracellular phosphorylation levels of Src and p38 following si-DLL1 transfection, Western blot was performed as described previously (23). Band intensities were measured by densitometry using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

GSE data-set analysis. Gene expression data (E-MTAB-6134, E-MEXP-2780, GSE17891, GSE21501, GSE57495, GSE62452, GSE71729, GSE79668, GSE84219, PACA-AU_PancreaticCancer-AU and PAAD-US-TCGA) with prognosis information were downloaded from Gene Expression Omnibus, ArrayExpress and ICGC data portal as previously described (24). Each probe was converted to EntrezID. Several probes for the same EntrezID were averaged. Quantile-quantile normalization was applied to all samples to remove batch effects. To test prognostic value of a gene, samples were divided into two groups using median gene expression level as threshold average value. Log-rank test was then performed using Graph Prism version 5.

Xenograft tumor model. Balb/c nude mice 6-8 weeks of age were obtained from Orient Bio (Seongnam, Korea). Panc-1 cells (1×10^7) were injected subcutaneously into the mouse flank and incubated for 40 days as described previously (25). Tumor shape was calculated as long/short axis of tumors. Tumor shape and body weight were recorded regularly. Animals were anesthetized by cervical dislocation and prepared tumor lysates for further WB analysis. Animal care and experiments were carried out in accordance with guidelines approved by the animal bioethics committee of Jeju National University (2016-0049).

Statistical analysis. Data are presented as means±standard deviation. Groups were compared using one-way analysis of variance with Tukey's *post hoc* test for significant main effects (SPSS 12.0K for Windows; SPSS Inc., Chicago, IL, USA). The correlation between DUSP28 expression and the expression levels of various mucins was analysed by Pearson's correlation coefficient (PCC) using GraphPad Prism version 5.01 for Windows (San Diego, CA, USA).

Results

Expression of Delta-like 1 (DLL1) in human pancreatic cancer. Expression profile of *DLL1* was obtained from the public microarray database GEO of human pancreatic cancer samples and normal pancreas. As shown in Figure 1A, *DLL1* mRNA levels were higher in pancreatic cancers than those of normal pancreas samples, although the difference was statistically insignificant. We next investigated protein the expression levels of DLL1 in separate human pancreatic cancer and normal pancreas tissues. *In vivo* expression levels of DLL1 were higher in pancreatic cancer tissues than those of normal pancreas (Figure 1B). In addition, we performed

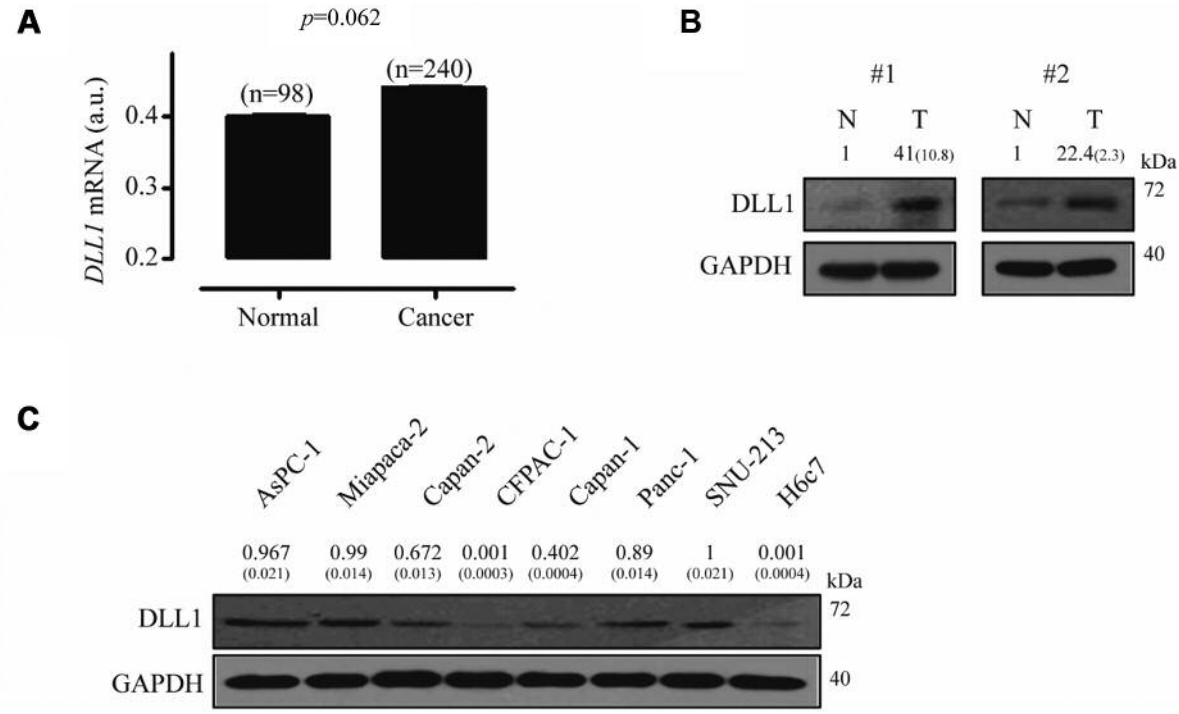

Figure 1. *Expression of Delta-like 1 (DLL1) in human pancreatic cancer. A: Transcriptional levels of DLL1 in pancreatic cancer and normal pancreas were analysed using the Gene Expression Omnibus (GEO) databases (p-values by Student's t test, a.u. indicate arbitrary units for the UPC method). B: Protein expression levels of DLL1 in pancreatic cancers and normal pancreas tissues were analysed by western blot. GAPDH was used as control (N indicates a normal pancreas sample, T indicates a pancreatic cancer sample, #1 and #2 are separate samples. Data are representative of three individual experiments Brackets indicate variance). C: Expression of DLL1 in seven human pancreatic cancer cells and H6c7 normal cells was determined by western blot analysis. GAPDH was used as a control. Relative pixel intensities for DLL1 were measured using the ImageJ analysis software. Data are representative of three individual experiments. Brackets indicate variance.*

western blot analysis using human pancreatic cancer cell lines and human normal pancreatic duct epithelial H6c7 cells. Expression patterns of DLL1 varied substantially. DLL1 was expressed relatively high in AsPC-1, Miapaca-2, Panc-1 and SNU-213. Its expression was moderate in Capan-2 and Capan-1. However, its expression was very weak or negative in CFPAC-1 and H6c7 cells (Figure 1C).

In vitro effects of Delta-like 1 (DLL1) silencing in human pancreatic cancer. To demonstrate the functional role of DLL1 expression in human pancreatic cancer, we performed transfection using si-RNA of DLL1 in human pancreatic cancer cells. Compared with scrambled siRNA-transfected AsPC-1, Capan-1, Capan-2 and SNU-213 cells, cells transfected with DLL1-specific siRNAs showed significantly decreased protein expression level of DLL1 (Figure 2A). Transfection with si-DLL1 had no effect on the viability of AsPC-1, Capan-1, Capan-2, Miapaca-2, Panc-1, SNU-213, or H6c7 cells (data not shown). In addition, the correlation between DLL1 expression and various anti-cancer drugs was not significant following the analysis of Cancer Cell Line Encyclopedia (CCLE) database (data not shown). By contrast, silencing DLL1 expression significantly increased the migration of AsPC-1, Capan-1, Capan-2, Miapaca-2, Panc-1 and SNU-213 cells (Figure 2B). However, there was no altered migration in H6c7 cells (data not shown). Ablating DLL1 expression also markedly stimulated invasion of AsPC-1, Capan-1, Capan-2, Miapaca-2, Panc-1 and SNU-213 cells compared with control (scrambled siRNA-transfected cells) (Figure 2C). These results indicate that DLL1 has a unique and functional role in migratory features of human pancreatic cancer cells.

Intracellular signalling by silencing of Delta-like 1 (DLL1) expression in human pancreatic cancer cells. To elucidate intracellular signalling of stimulated migratory features by silencing DLL1 expression in human pancreatic cancer cells, we investigated signal transduction mechanisms specifically regulated by transfection of scrambled si-RNA or si-DLL1 in Miapaca-2 and Panc-1 cells that were highly sensitive to si-DLL1 transfection. Transfection of si-DLL1 induced a dramatic increase in tyrosine phosphorylation of proteins

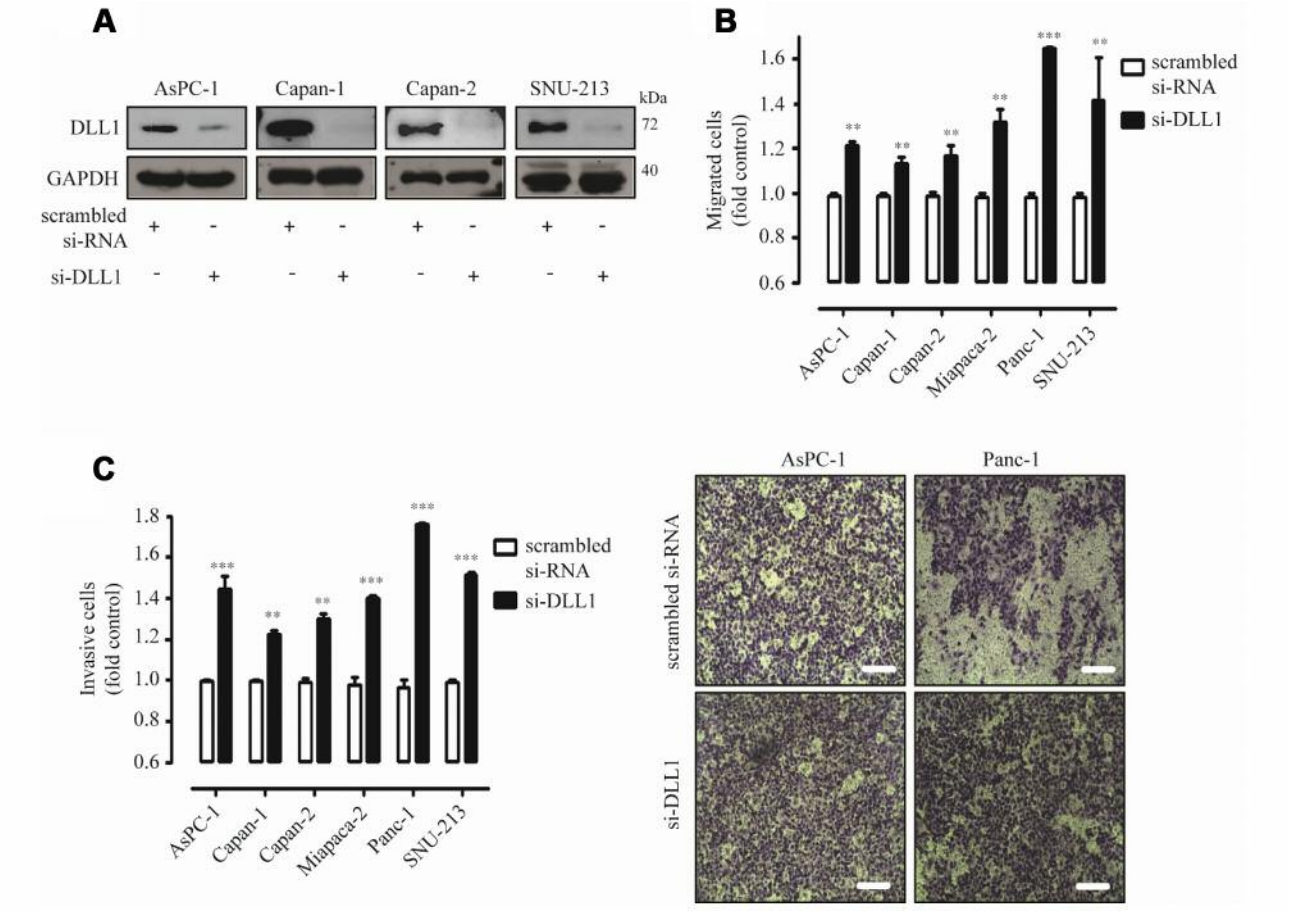

Figure 2. *In vitro effects of Delta-like 1 (DLL1) silencing in human pancreatic cancer. A: AsPC-1, Capan-1, Capan-2 and SNU-213 cells were transfected with scrambled or si-DLL1 for 72 h. DLL1 and GAPDH protein levels were analysed by western blot. Data are representative of three individual experiments. B: AsPC-1, Capan-1, Capan-2, Miapaca-2, Panc-1 and SNU-213 cells were transfected with scrambled or DLL1-specific siRNA. After 48 h of transfection, the cells were exposed to a serum-starved condition. After 18 h of serum-starvation, migrated cells were evaluated using the Transwell-migration assay (p-value by Student's t test, **p<0.01, ***p<0.001). C: Left, AsPC-1, Capan-1, Capan-2, Miapaca-2, Panc-1 and SNU-213 cells were transfected with scrambled or DLL1-specific siRNA. After 48 h of transfection, the cells were exposed to a serum-starved condition. After 18 h of serum-starvation, invasive cells were evaluated using the Transwell-invasion assay (p-value by Student's t test, **p<0.01, ***p<0.001). Right, Representative image of invasion assay using AsPC-1 and Panc-1 (scale bar=50 μm).*

with size of 38, 42, 60 and 120 kDa in Miapaca-2 and Panc-1 cells compared to transfection using scrambled si-RNA (Figure 3A). To further verify signal transduction pathways regulated by si-DLL1 transfections, we performed western blot analysis using antibodies against phosphor-FAKs (Tyr576/577 and Tyr397), phosphor-AKT (Ser473), phosphor-ERK1/2 (Thy202/204), phosphor-Src (Tyr416) and phosphor-p38 (Thr180/Tyr182). Transfection of si-DLL1 increased phosphor-Src and phosphor-p38 levels, but not notch1 intracellular domain (NICD). In addition, it did not regulate levels of phosphor-FAKs (Tyr576/577 and Tyr397), phosphor-AKT or phosphor-ERK1/2 level even at same transfection condition (Figure 3B). To confirm the involvement of Src and p38 pathway in the gain of function by ablating DLL1 expression, si-DLL1 transfected Miapaca-2 and Panc-1 cells were exposed in the absence or presence of a Src inhibitor (Src inhibitor-I; 1 μM) and a p38 inhibitor (p38 inhibitor IV; 1 μM) in migration assay. Treatment with Src inhibitor-I or p38 inhibitor IV differently inhibited the migration of Miapaca-2 and Panc-1 cells stimulated by si-DLL1 transfection. Of interest, migration following combined treatment of Src inhibitor-I and p38 inhibitor IV were almost the same as that after scrambled si-RNA transfection (Figure 3C). Similarly, combined treatment of Src inhibitor-I and p38 inhibitor IV markedly inhibited acquired invasion of Miapaca-2 and Panc-1 cells after si-

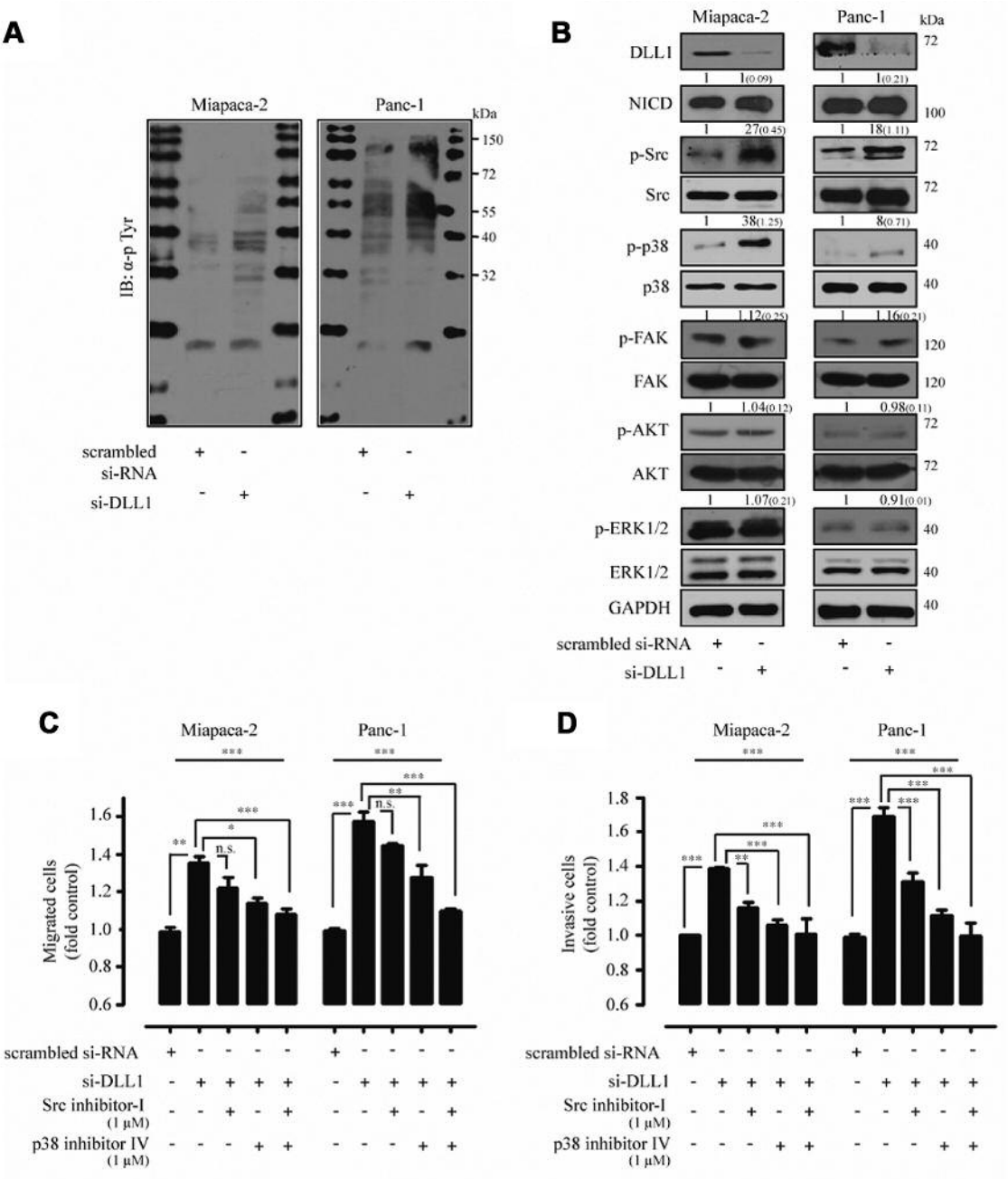

Figure 3. *Intracellular signalling by silencing Delta-like 1 (DLL1) expression in human pancreatic cancer cells. A: Miapaca-2 and Panc-1 cells were transfected with scrambled or DLL1-specific siRNA for 48 h. The cell lysates were prepared and analysed by western blot with antibody specific for phospho-tyrosine kinases (Y99). Data are representative of three individual experiments. B: Miapaca-2 and Panc-1 cells were transfected with scrambled or DLL1-specific siRNA for 48 h. The cell lysates were prepared and analysed by western blot with antibodies specific for DLL1, notch1 intracellular domain (NICD), phosphor-Src, Src, phosphor-p38, p38, phosphor-FAK, FAK, phosphor-AKT, AKT, phosphor-ERK1/2, ERK1/2 and GAPDH. Relative pixel intensities for DLL1 were measured using ImageJ analysis software. Data are representative of three individual experiments. Brackets indicate variance. C: Miapaca-2 and Panc-1 cells were transfected with scrambled or DLL1-specific siRNA. After 48 h of transfection, the cells were exposed to a serum-starved condition. After 18 h of serum-starvation, migrated cells exposed either in the absence or presence of Src inhibitor-I and p38 inhibitor IV (1 μM) were evaluated using the Transwell-migration assay (n=3; Tukey's post-hoc test was applied to detect significant differences in ANOVA, p<0.0001; asterisks indicate a significant difference compared with 0% inhibition, n.s., non-significant, **p<0.01, ***p<0.001). D: Miapaca-2 and Panc-1 cells were transfected with scrambled or DLL1-specific siRNA. After 48 h of transfection, the cells were exposed to a serum-starved condition. After 18 h of serum-starvation, invasive cells exposed either in the absence or presence of Src inhibitor-I and p38 inhibitor IV (1 μM) were evaluated using the Transwell-invasion assay (n=3; Tukey's post-hoc test was applied to detect significant differences in ANOVA, p<0.0001; asterisks indicate a significant difference compared with 0% inhibition, **p<0.01, ***p<0.001).*

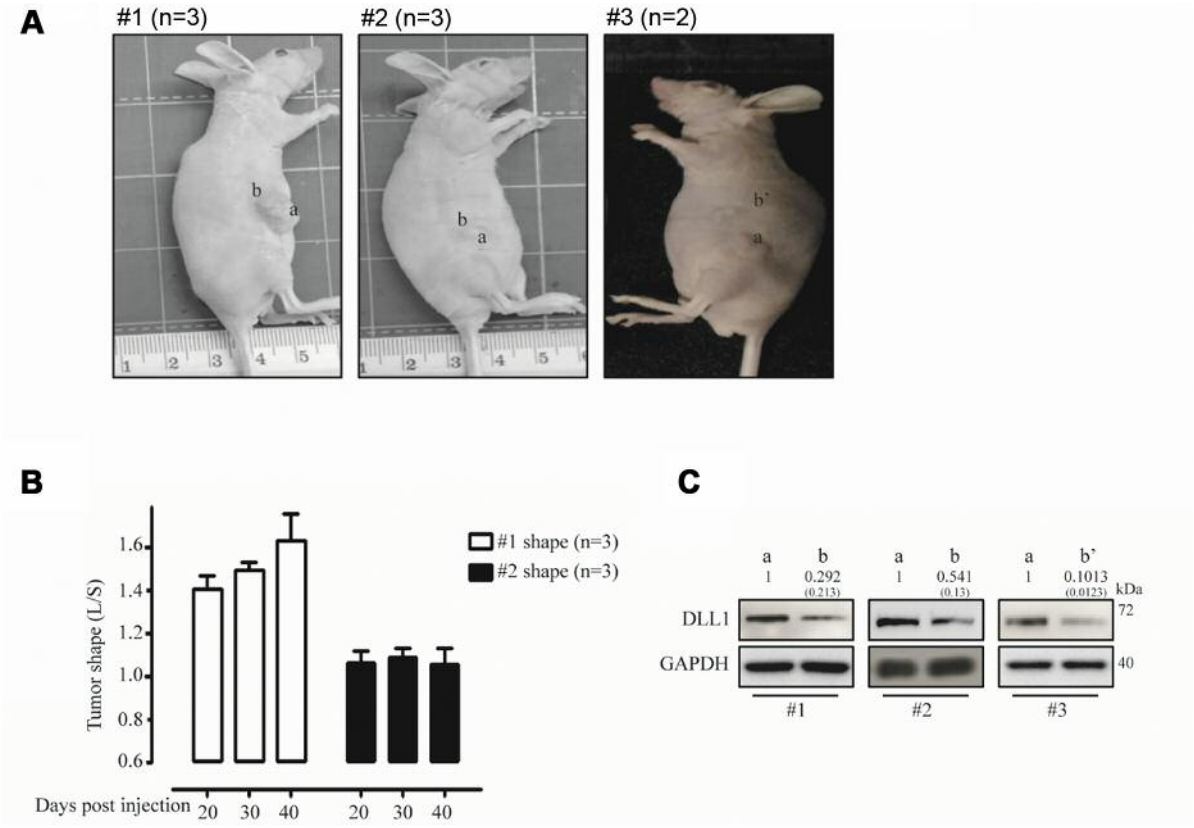

Figure 4. *In vivo expression of Delta-like 1 (DLL1) in human pancreatic cancer. A: Representative images of the Panc-1 xenograft models (#1, #2 and #3). (b) Indicates newly generated tumors from the tumor-headquarter in #3 Panc-1 xenograft models. B: Different tumor shape (#1 and #2) is determined to long/short axis of tumors. C: The lysates from Panc-1 xenograft tumor samples were tested for DLL1 and GAPDH by western blot analysis. Intensities of DLL1 expression in #1, #2 and #3 tumor models were measured using the ImageJ software. Data are representative of three individual experiments. Brackets indicate variance.*

DLL1 transfection (Figure 3D). These results suggest that DLL1 is associated with migratory features involving Src and p38 signalling pathway in human pancreatic cancer cells.

In vivo expression of Delta-like 1 (DLL1) in human pancreatic cancer. The correlation between DLL1 expression and migratory features of pancreatic cancer was observed in Panc-1 xenograft models using Western blot analysis. We divided the representative xenograft models into three groups (long tumor shape (n=3); #1, round tumor shape (n=3); #2 and newly migrated tumor (n=2); #3). We designated the core and peripheral regions of the tumor models as a and b (b' for #3), respectively (Figure 4A and 4B). Then, we performed the western blot analysis using whole fractions of #1, #2, and #3 samples from isolated tumor tissues. Expression levels of DLL1 were significantly decreased in b fractions compared to those of a fractions in #1, #2 and #3 samples (Figure 4C). No weight loss was detected in eight Panc-1 xenograft models (data not shown). These results indicate that migratory featured pancreatic cancer show the decreased DLL1 expression levels *in vivo*.

Patient prognosis was associated with Delta-like 1 (DLL1) expression in human pancreatic cancer. To determine the role of DLL1 expression in the prognosis of pancreatic cancer patients, we analysed GEO data-sets (E-MTAB-6134, E-MEXP-2780, GSE17891, GSE21501, GSE57495, GSE62452, GSE71729, GSE79668, GSE84219, PACA-AU_PancreaticCancer-AU and PAAD-US-TCGA) for overall survival (OS). GSE-21501, GSE-57495 and GSE71729 data-sets showed a functional DLL1 expression in pancreatic cancer prognosis by prolonging overall survival days of pancreatic cancer patients having high expression levels of DLL1. Data-set with an elevated expression of DLL1 showed better median survival than those expressing low levels of DLL1 (low DLL1: 425.83; high DLL1: 638.75

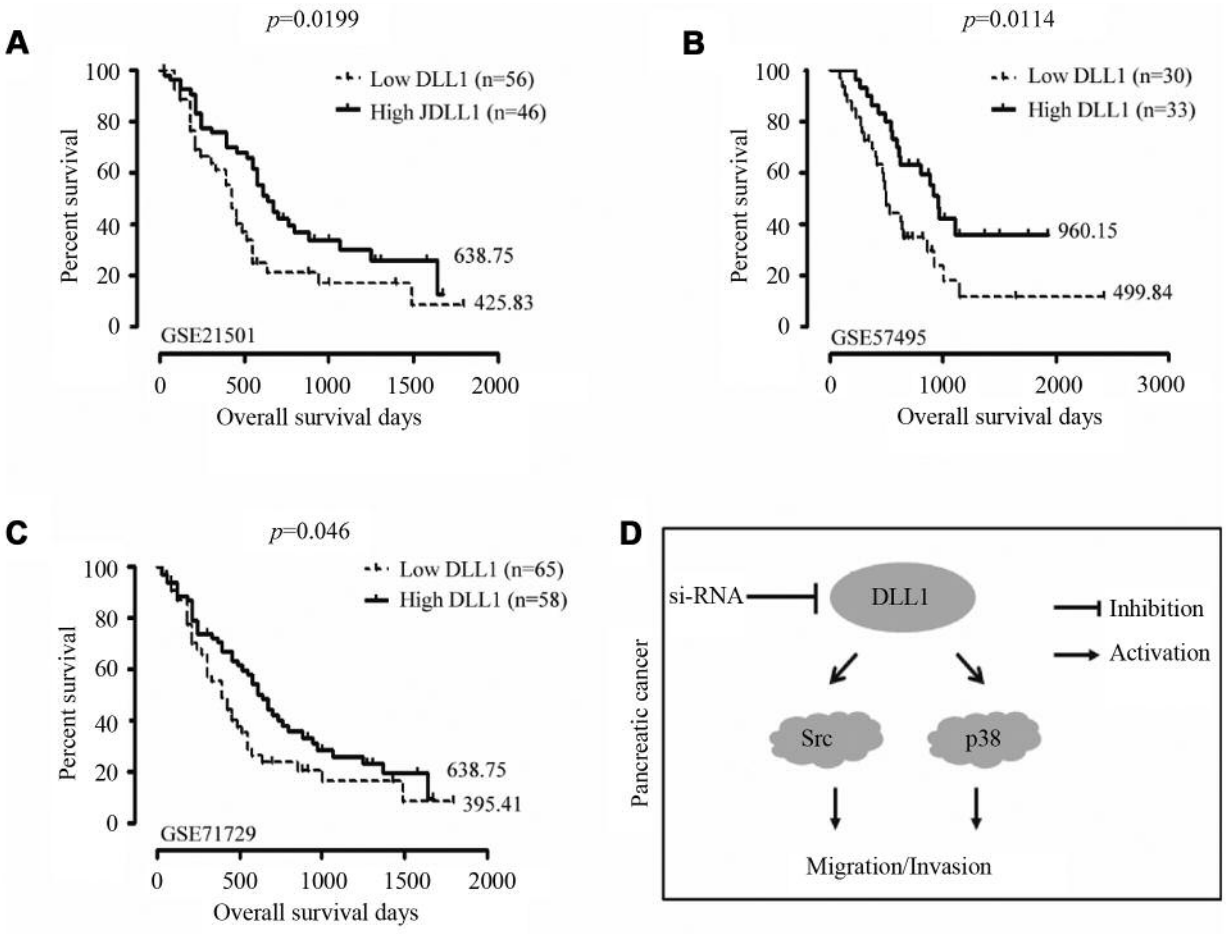

Figure 5. *Delta-like 1 (DLL1) is associated with prognosis of pancreatic cancer patients. A-C: Kaplan-Meier plot of the median survival for pancreatic cancer patients included dependent on differential expressions of DLL1 mRNA in GSE21501, GSE57495 and GSE71729 datasets (p-value was calculated using Log-rank (Mantel-Cox) Test). D: Action mechanism of silencing DLL1 expression in human pancreatic cancer.*

for GSE-21501, low DLL1: 499.84; high DLL1: 960.15 for GSE-57495, low DLL1: 395.41; high DLL1: 638.75 for GSE-71729) (Figure 5A-C). In addition, GSE62452, GSE79668, GSE84219, PACA-AU_PancreaticCancer-AU and PAAD-US-TCGA data-sets showed that enhanced expression of DLL1 prolonged overall survival days of pancreatic cancer patients than those expressing low levels of DLL1, though the difference was not statistically significant. In contrast, conflicting pattern was obtained from E-MTAB-6134, E-MEXP-2780 and GSE17891 data-sets, however, which was not statistically significant. These results indicate that high expression of DLL1 can improve the prognosis of pancreatic cancer patients.

Collectively, these results clearly indicate that functional DLL1 represents a suppressor of migratory features in pancreatic cancer involving the p38 and Src signalling pathway (Figure 5D).

Discussion

Although many researchers have developed innovative therapies and neoteric diagnostic techniques to remedy pancreatic cancer, the survival of pancreatic cancer patients has not improved during the last few decades (26, 27). Such poor prognosis reflects strong chemo-resistance, malignant metastasic nature and under-developed early diagnosis (28). Our study demonstrated that protein levels of Delta-like 1 (DLL1) were significantly enhanced in pancreatic cancer compared to those in normal pancreas both *in vitro* and *in vivo*. In addition, silencing DLL1 expression activated migratory natures in human pancreatic cancer cells through stimulation of p38 and Src signalling pathway. In addition, expression of DLL1 was correlated with migratory features of pancreatic cancer *in vivo* xenograft models. Moreover, high expression of DLL1 significantly improved pancreatic cancer prognosis. To

the best of our knowledge, this is the first report demonstrating that DLL is a potent suppressor of pancreatic cancer metastasis

Song and colleagues showed that Notch1, Notch2, DLL1, DLL3 and DLL4 were increased in pancreatic cancer *in vitro* and *in vivo*. Of interested, expression of DLL1 had the most highly positive rate in pancreatic cancer tissues compared to other Notch ligands. However, grade of pancreatic cancer patients involved the study was early stage such as grade1 and grade 2. Therefore, a supplementary study using late-stage pancreatic cancer tissues should be conducted to understand the expression level of DLL1 in pancreatic cancer (29). In agreement previous report, the current study also revealed a unique expression pattern of DLL1 in human pancreatic cancers. Based on western blot analysis, DLL1 was specifically over-expressed in pancreatic cancers compared with that in normal pancreas both *in vitro* and *in vivo*. However, further study should be performed to solve the inconsistency between mRNA and protein expression levels of DLL1 by up-dating information about expression profiles of DLL1 in pancreatic cancer through bioinformatics and to clarify the *in vitro* expression of DLL1 using more normal pancreas cells. In addition, expression of DLL1 in metastatic pancreatic cancer tissues should be analysed to complement the limitation of present study.

The function of Notch signalling in PDAC has been conflicting (30, 31). Initial reports have proposed that Notch signaling has oncogenic functions. However, blockade of Notch signalling has limited anti-cancer effects (9, 10). Moreover, recent reports have revealed that Notch signaling is a tumor-suppressor in PDAC development (11, 12). In agreement of previous reports emphasizing the tumor-suppressor function of Notch signalling, the present study revealed the DLL1 was a suppressor of the migratory nature of pancreatic cancer. Silencing of DLL1 significantly stimulated migration and invasion, but not viability of human pancreatic cancer cells. According to endogenous expression of DLL1 in seven pancreatic cancer cells, Capan-1 and CFPA254C-1 cells showed relatively low DLL1 expression levels compared to the other five pancreatic cancer cells. Capan-1 and CFPAC-1 cells were derived from metastatic site of liver as previously reported (32-35), implying expression level of DLL1 might be correlated with the natural metastatic character of pancreatic cancer cells as "signalling molecule". However, further studies such as soft agar assay, apoptosis assay, spontaneous metastatic *in vivo* models should be performed to complement the limitation of the present study for clarifying the function of DLL1 in pancreatic cancer.

Based on our results, we concluded that silencing DLL1 can stimulate the migratory nature of pancreatic cancer independent of classical Notch1 signalling pathway. Ablating DLL1 expression significantly induced the migration and invasion of AsPC-1, Capan-1, Capan-2, Miapaca-2, Panc-1 and SNU-213 cells. Notably, as shown Fig. 3B, notch1 intracellular domain (NICD) was expressed even after si-DLL1 transfection. Nevertheless, transfection of si-DLL1 altered features of pancreatic cancer cells, showing that their migratory nature stimulated by silencing DLL1 expression was advanced independent of classical Notch1 signalling pathway. However, further study should be performed to investigate the fundamental mechanism involved in the correlation between DLL1 expression level and pancreatic cancer migratory features.

Src and p38 signalling pathway has been found to have an oncogenic function in pancreatic cancer (36-38). Consistent with previous reports, results of the present study showed that silencing DLL1 expression increased phosphorylation of Src and p38 for the first time. By contrast, the acquired migratory nature of pancreatic cancer by ablating DLL1 expression was almost appeased following combined treatment with Src inhibitor-I and p38 inhibitor IV. It is noteworthy that the functional activity of Src and p38 signalling pathway is regulated by DLL1 expression level, indicating that DLL1 expression affects post-transcriptional processes of pancreatic cancer, especially metastatic features.

In vivo evaluation of DLL1 expression levels in migratory featured pancreatic cancer has been observed in Panc-1 xenograft models in sub-divided three groups as the tumor shape (#1:n=3, #2:n=3 and #3:n=2). Importantly, Expression levels of DLL1 were clearly decreased in migratory featured pancreatic cancer compared to those of non-migrated pancreatic cancer, implying the functional role of DLL1 expression as a suppressor of metastasis in pancreatic cancer. The findings may facilitate patient-specific strategies monitoring the metastasis of pancreatic cancer. Further study using various human tissues originated from pancreatic cancer patients should be performed to strongly understand the correlation between DLL1 expression levels and pancreatic cancer metastasis.

Previous reports have suggested that DLL1 expression is significantly associated with prognosis of various cancers (13, 39, 40). However, the clinical significance of DLL1 expression in pancreatic cancer prognosis remains ambiguous. The present study revealed that high expression of DLL1 significantly improved median survival compared to high expression of DLL1 in patients with pancreatic cancer in GSE-21501, GSE57495 and GSE71729 datasets. Similar patterns were obtained from GSE62452, GSE79668, GSE84219, PACA-AU_PancreaticCancer-AU and PAAD-US-TCGA data-sets, though the difference was not statistically significant. To the best of our knowledge, this is the first study showing that DLL1 expression has clinical significance in human pancreatic cancer. In addition, continuous analysis should be performed by up-dating information involving prognosis to expression profiles of DLL1 in E-MTAB-6134, E-MEXP-2780 and GSE17891 data-sets.

Collectively, our results provide insight into DLL1 expression-related regulation of pancreatic cancer migratory features, showing that it is a potent suppressor of pancreatic cancer metastasis. Understanding correlation between expression and function of DLL1 might contribute to our knowledge of the complicated mechanism of pancreatic cancer metastasis.

Conflicts of Interest

There are no potential conflicts of interest regarding this study.

Authors' Contributions

JW. Lee designed the study and drafted the manuscript. JW. Lee and D. Kim performed *in vitro* and *in vivo* experiments. J. Lee arranged all data-sets. JW. Lee and J.H. Kim supervised the project. All Authors discussed data and read the manuscript.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1D1A1B03033899) and (2016R1A6A1A03012862).

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Received January 23, 2020
Revised January 29, 2020
Accepted February 3, 2020