DJ-1 promotes invasion and metastasis of pancreatic cancer cells by activating SRC/ERK/uPA

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Introduction
Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal forms of cancer. It is the 13th most frequently diagnosed cancer worldwide (1) and the 8th cause of cancer mortality. It is responsible for almost a quarter of a million deaths each year (1), with an overall cumulative 5 years survival rate <5% (2). The high mortality rate is due to extensive local tumor invasion and early systemic dissemination. Only 5–25% of the patients presenting with pancreatic cancer will have an operable tumor (3). It is, therefore, critical to understand the mechanisms underlying pancreatic cancer invasion and metastasis processes.

DJ-1 is a 189 amino acid protein with multiple functions. It was originally identified as an oncogene that can transform mouse mouse embryonic fibroblast; MMP, matrix metalloproteinase; NC shRNA, scrambled sequence of the shRNA target sequence; containing the green fluorescent protein expression sequence were purchased from Gempharma (Shanghai, China). The target of DJ-1 shRNA was S'–GGTCTGGTGGCTCTACTAAA–3'. The pCMV6-XL5-DJ-1 plasmid was obtained from OriGene (Rockville, MD). The shRNA-resistant DJ-1 expression vector, pCMV6-DJ-1, was constructed from pCMV6-XL5-DJ-1 by using the QuickChange® Lightning Site-Directed

Abbreviations: ECM, extracellular matrix protein; ERK, extracellular signal-regulated kinase; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; MEF, mouse embryonic fibroblast; MMP, matrix metalloproteinase; NC shRNA, negative control shRNA; PAI, plasminogen activator inhibitor; PDAC, pancreatic ductal adenocarcinoma; NC shRNA, negative control shRNA; PTEN, phosphatase and tensin homolog; shRNA, short hairpin RNA; uPA, urokinase plasminogen activator; uPAR, uPA receptor.

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Mutagenesis Kit (Stratagene, La Jolla, CA) according to the manufacturer’s instructions and using the following primers: DJ-1 shRNA-mutation1 forward 5’-GCAAGGTCTCCTGCTCTGCTGGCAGAAATTAGGTGGTGTTGG-3’ (the mutated sites were underlined) and DJ-1 shRNA-mutation1 reverse 5’-GCACCAAACTTTGCTGCTACAGCAGGAAGCATAGGGACCTGC-3’. ERK1/2 small interfering RNA (siRNA) was purchased from Cell Signaling Technology (Danvers, MA). Cells were transfected with vectors using Lipofectamine 2000TM (Invitrogen), followed by one change of media, according to the manufacturer’s instruction, using the recommended concentration of plasmid and/or siRNA. To generate stable shRNA knockdown cells, after 2 days of transfection, cells were selected in media containing 1000 μg/ml G418 (Promega, Madison, WI) for 4 weeks. Stably transfected clones were recovered and maintained in medium containing 500 μg/ml G418 for further study.

Western blotting analysis

Western blotting analysis was performed as described previously (19). Briefly, cell lysates were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a polyvinylidifluoride membrane. After blocking of non-specific binding sites with 5% fat-free milk, the blot was then probed with primary antibodies (see Supplement Materials, available at Carcinogenesis Online). The bound antibodies were detected by horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and visualized using an enhanced chemiluminescence system (Millipore, Billerica, MA). The levels of each protein were standardized to the loading control, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and were quantified using an Image J 1.41 program (National Institutes of Health, Bethesda, MD). For phosphorylated protein quantification, the same amounts of total protein were loaded onto two separate gels for sodium dodecyl sulfate–polyacrylamide gel electrophoresis and western blotting analysis. The proportions of phosphorylated proteins were calculated as phosphoprotein/GAPDH, total protein/GAPDH and phosphoprotein/total protein = [phosphoprotein/GAPDH]/total protein/GAPDH.

Immunohistochemistry

Immunohistochemical staining was carried out as described previously using a Dako EnVision + System horseradish peroxidase (20). DJ-1 polyclonal antibody from Abcam (ab18257; Cambridge, MA) was applied at a 1:2000 dilution and incubated overnight at 4°C. The primary antibody was omitted in the negative control. The stained sections were reviewed by two independent observers (Z.Z. and X.Y.H.), who had no prior knowledge of the clinical pathologic data of the patients. A scoring method was used as previously reported, based on the fact that the specimens clearly showed a varying degree of staining intensity and percentage of cell staining (21). Briefly, strong-intensity staining was scored as 3, moderate as 2, weak as 1 and negative as 0. For each intensity score, the percentage of cells with that score was estimated visually. A combined weighted score consisting of the sum of the percentage of cells staining at each intensity level was calculated for each sample. The immunolabeling was categorized as negative (score ≤ 30) or positive (score > 30) for all the tissues.

![Fig. 1](image-url) DJ-1 expression in pancreatic cancer and correlation with tumor stage and progression. (A) Representative images of immunohistochemical staining of DJ-1 in pancreatic cancer (top) and non-cancerous pancreatic tissue (middle). The isotype control was shown at the bottom. The magnification used is indicated at the left top of each image. (B) Staining of DJ-1 and correlation with tumor stage (P = 0.02, determined by Mann–Whitney U-test). (C) Kaplan–Meier analysis of the significance of DJ-1 in predicting PDAC patient overall survival.
Migration and invasion assay

Cell invasion was determined by using a modified two-chamber migration assay (8 μm pore size; BD Biosciences, Sparks, MD) or invasion assay [membrane coated with a layer of Matrigel extracellular matrix proteins (ECM): BD Biosciences] according to the manufacturer’s instructions. Cells were seeded at a density of 2.5 × 10^5 in serum-free medium into the upper chamber and allowed to migrate/invade toward the chemoattractant 10% fetal calf serum in the lower chamber for 20 h (for migration assay) or 48 h (for invasion assay). Cells in the upper chamber were then carefully removed using cotton buds, and cells at the bottom of the membrane were fixed and stained with 0.1% crystal violet in methanol. Quantification was performed by manually counting the stained cells.

uPA activity and enzyme-linked immunosorbent assay

Serum-free media supernatant was collected from cells after 24 h incubation and analyzed for uPA activity by using the uPA Activity Assay Kit (Chemicon, Temecula, CA) according to the manufacturer’s instructions. Briefly, supernatant aliquots were combined with assay buffer in 96-well plates. After a 24 h incubation of the mixture with a chromogenic substrate (1:1 by volume) at 37°C, absorbance was read at 405 nm (SpectroMax 190; Molecular Devices, Sunnyvale, CA) and converted to units of activity using standard curves generated with the pure uPA enzyme provided with the kit. Activity was expressed per milligram of total sample protein, as determined by a BCA Protein Assay Kit (Pierce, Rockford, IL).

In vivo experimental metastasis assay

Human pancreatic cancer cells were injected (2 × 10^6/100 μl of phosphate-buffered saline) into tail veins of male nude mice (4–6 weeks old). After 8 weeks, the mice were killed and the lungs and liver were surgically excised and examined for metastatic lesions under a dissecting microscope (×40 magnification). Microscopic quantification of metastases was performed on representative lung cross-sections of formalin-fixed paraffin-embedded tissues stained with hematoxylin and eosin. The total area of lung metastasis and the total area of the lungs were measured for each animal by using a microscope (Nikon, Tokyo, Japan) and imaging software (ACT-2U Imaging Software, Enfield, CT).

Statistical analysis

Data are represented as mean ± SD from at least three independent experiments or as median ± SEM, as indicated. Data were analyzed by the Student’s t-test, chi-square test or analysis of variance as appropriate and by using the SPSS v14.0 statistical program (Chicago, IL). A two-tailed P-value <0.05 was considered to be statistically significant. Survival data were analyzed using the Kaplan–Meier method with a log-rank test for comparison of survival curves. Cox’s proportional hazards regression was used for multivariate analysis.
Results

DJ-1 is overexpressed in PDAC and correlates with tumor invasion and poor prognosis

In cohort 1, 86% (66/76) of PDAC specimens were positively stained with anti-DJ-1, according to our criteria (see Materials and methods), whereas only 34.2% (26/76) of non-neoplastic specimens showed DJ-1-positive staining ($P$, 0.05). In normal pancreatic tissues, the expression of DJ-1 was mainly localized in nuclei of ductal and acinar cells. In PDAC specimens, DJ-1 staining occurred in both the cytoplasm and nuclei. When compared with matched controls, 50 (68.5%), 5 (6.6%) and 21 (27.6%) cases showed higher, equal and lower total DJ-1 expression, respectively. When nuclear or cytoplasmic DJ-1 was analyzed alone, both were found to be significantly upregulated in pancreatic cancer (for both, $P < 0.05$). In normal pancreatic tissues, the expression of DJ-1 was mainly localized in nuclei of ductal and acinar cells. In PDAC specimens, DJ-1 staining occurred in both the cytoplasm and nuclei. When compared with matched controls, 50 (68.5%), 5 (6.6%) and 21 (27.6%) cases showed higher, equal and lower total DJ-1 expression, respectively. When nuclear or cytoplasmic DJ-1 was analyzed alone, both were found to be significantly upregulated in pancreatic cancer (for both, $P < 0.001$). The upregulation of DJ-1 in pancreatic cancer was also confirmed by western blotting analysis in four cases (Supplementary Figure S1 is available at Carcinogenesis Online). There was no difference in the correlation between DJ-1 expression and age, gender or tumor differentiation grade, as shown in Supplementary Table S1, available at Carcinogenesis Online. Because there was no other clinical or pathological information available for these 76 cases, we examined DJ-1 expression in a second cohort of 85 PDAC cases, for which the TNM stage and follow-up information were available. DJ-1 was overexpressed in 89.4% (76/85) of these PDAC specimens (Figure 1A), having a similar expression pattern to the previous tissue array. The correlation of DJ-1 expression with clinical parameters is shown in Supplementary Table S2, available at Carcinogenesis Online. Higher DJ-1 expression was correlated with higher T stage tumor (T3/T4; Figure 1B) but not N or M stage. In addition, DJ-1 expression in PDAC was correlated with a shorter overall survival (Figure 1C). However, Cox regression analysis indicated that the result was not statistically significant ($P = 0.083$).

DJ-1 regulates pancreatic cancer cell migration and invasion

DJ-1 was expressed in the two pancreatic cancer lines, BxPC-3 and SW1990, as detected by western blotting analysis (Figure 2A). We sought to investigate whether DJ-1 had a direct functional role in facilitating tumor cell migration and invasion in pancreatic cancer. Therefore, we evaluated cancer cell invasion through Matrigel and migration through a transwell, after knockdown of DJ-1 expression. shRNA was used to induce stable knockdown of DJ-1 expression in BxPC-3 and SW1990 cells. As measured by western blotting analysis (Figure 2A) and reverse transcription–PCR (Supplementary Figure S2
is available at Carcinogenesis Online). DJ-1 expression levels in cells transfected with shRNA targeting DJ-1 (BxPC-3/DJ-1 shRNA, SW1990/DJ-1 shRNA) were significantly reduced, as compared with control cells (transfected with NC shRNA, BxPC-3/NC shRNA or SW1990/NC shRNA). Knockdown of DJ-1 in BxPC-3 and SW1990 cells led to a significant decrease in both cell invasion and migration, as compared with control cells (Figure 2B–E).

To confirm that the reduced invasion and migration was not due to non-specific effects, we restored DJ-1 expression in the two cell lines with DJ-1 shRNA by transfection of a shRNA-resistant DJ-1 vector (pCMV-rDJ-1). The target of shRNA in pCMV-rDJ-1 was mutated without changing the amino acid sequence. After transient transfection of pCMV-rDJ-1 for 24 h, DJ-1 expression in BxPC-3/DJ-1 shRNA and SW1990/DJ-1 shRNA was restored to a level higher than baseline level, whereas transfection with the pCMV-DJ-1 vector slightly increased DJ-1 levels due to the inhibition of expression by DJ-1 shRNA (Figure 2A). As expected, restoration of DJ-1 expression restored both cell invasion and migration (Figure 2B–E) in both pancreatic cancer cell lines. In contrast, transfection of NC shRNA PDAC cells with pCMV-DJ-1 or pCMV-rDJ-1 increased DJ-1 level by 2-fold and also increased the invasion cell number by 20–40% (P < 0.05), as compared with transfection of NC shRNA PDAC cells with pCMV. Ectopic expression of DJ-1 in NC shRNA pancreatic cancer cells also increased cell migration, as compared with control cells.

The reduction in invasion and migration potential by knockdown of DJ-1 was not attributable to induction of apoptosis or retardation of cell growth because in the cell proliferation (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay), cell apoptosis and cell cycle (DNA content analyzed by flow cytometry) and cellular colony formation analyses, there were no differences between the DJ-1-silenced cells and control cells (Supplementary Figures S3 and S4 are available at Carcinogenesis Online).

Silencing of DJ-1 reduced uPA expression and activity and induced cytoskeleton disruption

To understand the mechanism underlying DJ-1 regulation of cell invasion, we measured uPA and Matrix metalloproteinases (MMPs) 2 enzyme activity. Downregulation of DJ-1 expression resulted in decreased uPA activity, which was reversed by restoration of DJ-1 expression (Figure 3A), and was increased further by ectopic expression of DJ-1 in NC shRNA PC cells. Since uPA is activated by the uPA receptor (uPAR) and inhibited by plasminogen activator inhibitor (PAI), we also analyzed uPAR and PAI-1 expression. However, the expression of uPAR and PAI-1 remained unchanged after knockdown of DJ-1 expression (Figure 3B), but the uPA protein levels in cells and culture supernatants were decreased, as determined by Western blot (Figure 3B) and enzyme-linked immunosorbent assay, respectively (Figure 3C). In addition, plasmin activated by uPA can break down ECM directly or degrade the ECM indirectly through activation of pro-MMPs (such as, pro-MMP2) (22,23). Zymography revealed that MMP2 was the major form of MMPs, which was abundantly secreted by BxPC-3. Silencing of DJ-1 by shRNA resulted in reduction of MMP2 activity (Supplementary Figure S5 is available at Carcinogenesis Online). However, in SW1990 cells, the MMP2 activity was undetectable in the culture media (data not shown). The protein levels of MMP2, MMP9, Tissue inhibitor of metalloproteinase-1 and Tissue inhibitor of metalloproteinase-2 in the cultured media remained unchanged after knockdown of DJ-1 expression (data not shown).

Because the cytoskeleton is an important component required for cell mobility, we aimed to determine whether the effect of DJ-1 on cell migration involved the cytoskeleton. As shown in Supplementary Figure S6, available at Carcinogenesis Online, regular arrays of large actin stress fibers were visible in parental cells and control cells transfected with NC shRNA. In contrast, DJ-1 knockdown resulted in severe disruption and decreased actin stress fibers.


**DJ-1 regulates the SRC and ERK pathways**

It has been reported that DJ-1 is a negative regulator of Phosphatase and tensin homolog (PTEN) (12) and protects cells from apoptosis by activating the Akt pathway (12,15,24). We determined whether DJ-1 was capable of promoting migration and invasion via the Akt pathway in these pancreatic cancer cells. Unexpectedly, no alteration of Akt phosphorylation levels was observed after knockdown of DJ-1 expression (Supplementary Figure S7 is available at Carcinogenesis Online). It has been reported that in Pten+/− mouse embryonic fibroblasts (MEFs), DJ-1 underexpression results in decreased phosphorylation of Akt, but the same effect is not present in Pten−/− MEFs (12). Both SW1990 and BxPC-3 express wild-type PTEN (determined from the COSMIC database). However, in the current study, PTEN expression was undetectable in either cell line by western blotting analysis (Supplementary Figure S8 is available at Carcinogenesis Online). Since the mitogen-activated protein kinase pathway has been implicated in cancer cell invasion, we examined the mitogen-activated protein kinase pathway in these PC cells. Interestingly, knockdown of DJ-1 expression in BxPC-3 and SW1990 resulted in decreased ERK1/2 and SRC phosphorylation (Figure 4A and B), without affecting c-jun N-terminal kinase (Supplementary Figure S7 is available at Carcinogenesis Online). Furthermore, P38 phosphorylation remained undetectable (data not shown). The reduction of ERK1/2 and SRC phosphorylation was reversed by restoration of DJ-1 expression and could be increased further by ectopic expression of DJ-1 in NC shRNA pancreatic cancer cells (Figure 5C; Supplementary S9 is available at Carcinogenesis Online). To further determine whether the effects of DJ-1 on cell invasion and migration were dependent on the ERK pathway, cell invasion and migration was measured following inhibition of ERK by U0126. Inhibition of ERK had the same effects as knockdown of DJ-1, with a reduction of pancreatic cancer cells invasion potential (Supplementary Figure S10A is available at Carcinogenesis Online) and cell migration (Supplementary Figure S10B is available at Carcinogenesis Online), a decrease in uPA expression (Supplementary Figure S10C is available at Carcinogenesis Online) and activity (Supplementary Figure S10D is available at Carcinogenesis Online) and induced cytoskeleton disruption (Supplementary Figure S11C is available at Carcinogenesis Online) in a dose-dependent manner. However, there was no effect on PAI-1 and uPAR (Supplementary Figure S10C is available at Carcinogenesis Online). In
Knockdown of DJ-1 inhibits pancreatic cancer tumor metastasis in vivo

To confirm the role of DJ-1 in invasiveness in vivo, pancreatic cancer cells with or without endogenous DJ-1 knockdown were injected into nude mice via the tail vein. The cells with endogenous DJ-1 formed colonies in the lungs within 2 months of injection, whereas the DJ-1 knockdown resulted in significantly less pulmonary metastatic colonization, both in number and size (Figure 6).

Discussion

DJ-1 has been reported as being upregulated in a number of different cancer types (8,12,13,17). Mei et al. showed that 72.5% of PADC tissues were DJ-1 positive by immunohistochemistry and that DJ-1 expression was significantly higher than that in normal pancreas tissues. This was similar to our results, which showed that 88.2% (142/161) of PDAC were positive and that 68.5% of PDAC specimens had upregulated DJ-1 expression. However, the prognostic significance of DJ-1 in PDAC has not yet been reported. Our results showed that the percentage of positive DJ-1 staining was increased along with tumor stage. Tumor stage was classified based on tumor size and invasion. Since there was no association of tumor size with DJ-1 expression, DJ-1 was mainly correlated with tumor invasion. Another finding of our study was that DJ-1 expression in PDAC specimens predicted poor survival. However, because tumors are seldom surgically resected when metastasis is already established, only four cases with distant metastasis were included in this study. This may have caused a negative result in the determination of the difference of DJ-1 levels between metastasis and non-metastasis specimens.

The ability to migrate and invade the basement membrane into surrounding tissues, blood and lymphatic vessels is one of the essential hallmarks of cancer and is a prerequisite for local tumor progression and metastatic spread (25). We have shown here that DJ-1 regulates pancreatic cancer cells migration and invasion properties in cell culture. In addition, our data indicated that reduction in DJ-1 expression severely impaired the ability of pancreatic cancer cells to form lung colonies in an in vivo experimental metastasis model. Extensive studies have shown that DJ-1 plays an important role in cell survival (12,15,26); however, our in vitro assay indicated that cell survival remained unchanged upon knockdown of DJ-1 expression. This suggested that the ability of DJ-1 to promote metastasis is mainly due to its effects on regulation of PDAC cell migration and invasion. The essential requirement for DJ-1 in pancreatic cancer cell migration and invasion highlights the potential for using DJ-1 as a target for blocking PDAC local invasion.

Our studies also point to mechanisms by which DJ-1 modulates migration invasion. Polymerization of actin to generate protrusive activity at the cell front (anterior), coupled with actin-myosin filaments generating contraction at the sides and rear (posterior), provide the major driving forces for migration (27). We have shown that DJ-1 silencing resulted in disruption of actin stress fibers. Therefore, we propose that in cells with organized actin cytoskeleton, DJ-1 is required for cell migration. However, further study is needed to address the underlining mechanism and how DJ-1 is able to regulate the actin cytoskeleton without affecting cell proliferation. Cancer invasion and metastasis require controlled degradation of the ECM. uPA and certain MMPs have been shown to be upregulated in cancer cells and to play a critical role in these processes (22,28). At the leading edge of migrating cells, the uPAR binds inactive uPA, which is then converted to active uPA. Active uPA proteolytically converts inactive plasminogen to active plasmin, which then breaks down ECM or activates growth factors, such as transforming growth factor β1. Plasmin can also degrade the ECM indirectly through activation of pro-MMPs (such as, pro-MMP2) (22,23). In both BxPC-3 and SW1990 cells, knockdown of DJ-1 resulted in reduction of uPA activity without changes in PAI-1 and uPAR expression. In BxPC-3, we have also shown that DJ-1 regulates MMP2 activity. However, in SW1990, endogenous MMP2 activity was undetectable. We propose that the uPA system is involved in DJ-1 promotion of cell invasion. MMP2 may also be involved in certain types of pancreatic cancer cells.

In order to understand the connection between DJ-1 and pancreatic cancer cells invasion/migration in more detail, it will be of great interest to identify signaling cascades by which DJ-1 regulates invasion/migration. Our results, for the first time, demonstrate that invasion by DJ-1-mediated pancreatic cancer cells is independent of the Akt pathway but dependent on the SRC/ERK pathway. We demonstrated that inhibition of the ERK pathway could explain the effects of DJ-1 silencing on migration and invasion. This is in contrast to previous work that proposed DJ-1 to be a potent modulator of the PI3K/Akt pathway in both cancer cells and neurons (12,15,26); however, our experimental metastasis model. Extensive studies have shown that DJ-1 plays an important role in cell survival (12,15,26); however, our in vitro assay indicated that cell survival remained unchanged upon knockdown of DJ-1 expression. This suggested that the ability of DJ-1 to promote metastasis is mainly due to its effects on regulation of PDAC cell migration and invasion. The essential requirement for DJ-1 in pancreatic cancer cell migration and invasion highlights the potential for using DJ-1 as a target for blocking PDAC local invasion.

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may have left phosphorylated Akt levels unchanged after DJ-1 silencing. In addition, previous studies showing that DJ-1 is associated with Akt were all restricted to the survival pathway (12,15,24,29). Whether DJ-1 utilized the same pathway for its effects on cell motility and invasion is an interesting question. Several lines of study have already shown that the ERK1/2 pathway plays an important role in PDAC metastasis (31,32). Indeed, DJ-1 was first observed to interact with the ERK1/2 pathway in neurons (26,33,34). All these previously published findings support our hypothesis that DJ-1 regulates invasion/migration via the ERK1/2 pathway. However, the means by which DJ-1 regulates ERK1/2 remains unknown. Our unpublished data showed that DJ-1 regulates RAS activity. However, whether RAS or RAS mutation is involved in the process requires further study.

In summary, DJ-1 is correlated with PDAC local invasion and promotion of cell invasion and migration. The effects are dependent on the SRC/ERK/uPA pathway. This suggests that DJ-1 is a predictor of PDAC invasion and may be a potential therapeutic target for blocking PDAC invasion.

Supplementary material

Supplementary Materials, Figures S1–S11 and Tables 1 and 2 can be found at http://carcin.oxfordjournals.org/

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References