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Original contribution

Overexpression of DIXDC1 correlates with enhanced cell growth and poor prognosis in human pancreatic ductal adenocarcinoma☆

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Summary Disheveled-axin (DIX) domain containing 1 (DIXDC1), a protein containing a coiled-coil domain and a DIX domain, is involved in the progression of multiple cancers. However, the role of DIXDC1 in human pancreatic ductal adenocarcinoma (PDAC) remains unclear. In this study, we investigated the role and prognostic value of DIXDC1 in the development of human PDAC. Western blot analysis revealed that DIXDC1 was highly expressed in PDAC tissues and cell lines. Immunohistochemistry on 165 paraffin-embedded sections showed that high expression of DIXDC1 was significantly correlated with tumor size ($P = .002$), histological differentiation ($P = .001$), tumor node metastasis (TNM) stage ($P = .001$), and the proliferation marker Ki-67 ($P = .000$). Importantly, Kaplan-Meier analysis revealed that high expression of DIXDC1 was obviously correlated with worsened overall survival ($P < .001$). In vitro, using serum starvation–refeeding experiments, our results suggested that DIXDC1 was up-regulated in proliferating PDAC cells, together with the percentage of cells at the S phase, and was correlated with the expression of cyclin D1. In addition, depletion of DIXDC1 decreased PCNA and cyclin D1 levels. Accordingly, CCK-8, colony formation, and flow cytometry analyses revealed that knocking down DIXDC1 induced growth impairment and G1/S cell cycle arrest in PDAC cells, while overexpression of DIXDC1 led to accelerated cell proliferation and cell cycle progression. On the basis of these results, we propose that DIXDC1 could play an important role in the tumorigenesis of PDAC and serve as a potential therapeutical target to prevent PDAC progression.

1. Introduction

Pancreatic cancer is the fourth leading cause of cancer-related death in developed countries with a dismal overall 5-year survival rate of 5% [1,2]. Pancreatic ductal adenocarcinoma (PDAC)

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accounts for 95% of pancreatic cancer and has a dismal prognosis [3,4]. The poor prognosis is mainly attributed to late diagnosis and resistance to chemotherapy [5]. Although intensive investigations have been done to develop advanced diagnostics and new treatment of PDAC, many aspects of the exact molecular mechanisms underlying PDAC remain to be clarified [6]. Therefore, there is an urgent need to find novel valuable prognostic biomarkers and therapeutic targets for early diagnosis and effective treatment of PDAC.

Disheveled-axin (DIX) domain containing 1 (DIXDC1) is a protein containing a coiled-coil domain and a DIX domain. DIXDC1 has been identified as a positive regulator in the Wnt signaling pathway [7]. It has been demonstrated that the DIX domain is important in connecting the Wnt-signaling factors Axin, Disheveled and β-catenin [8-10]. In the Wnt signaling pathway, the DIX domain is involved in the initiation of both homomeric and heteromeric complexes between Axin and Disheveled (Dvl) that eventually form the multiprotein complexes of adenomatous polyposis coli, glycogen synthase kinase 3β, and β-catenin, which regulate T-cell-factor signaling [9,11]. Despite the fact that the importance of DIX domain in the transduction of Wnt signaling has been well-confirmed, the role of DIXDC1 in human cancer development remains poorly understood. In colon cancer, up-regulation of DIXDC1 might target p21 and cyclin D1 to promote cell proliferation and tumorigenesis through activating the PI3K/Akt pathway [11]. In non–small cell lung cancer, overexpression of DIXDC1 promoted tumor invasion and migration through PI3K-Akt/AP-1–dependent activation of metalloproteinases [12]. In gastric cancer, up-regulated expression of DIXDC1 promoted cell invasion and metastasis by activating the Wnt signaling pathway [13]. These studies imply that DIXDC1 may be involved in cell proliferation and invasion during the development of multiple tumors. However, the expression and pathological significance of DIXDC1 in pancreatic cancer remain obscure.

In this study, we for the first time demonstrated that DIXDC1 was significantly overexpressed in human PDAC specimens and cell lines. Meanwhile, we explored the correlation of DIXDC1 with various clinical and pathological parameters as well as the prognosis in patients with PDAC using immunohistochemistry and Western blot analyses. Moreover, we employed small interfering RNA (siRNA) oligos and flag-DIXDC1to further explore the role of DIXDC1 in regulating cell cycle progression and cell proliferation in PDAC. These results showed that DIXDC1 might be a novel prognostic marker and play a potential role in anti-proliferative therapy of PDAC.

2. Materials and methods

2.1. Patients and tissue samples

The paraffin-embedded pathologic specimens from 165 patients with PDAC were obtained from the Surgery Department, at the Affiliated Hospital of Nantong University. All patients underwent surgery without preoperative systemic chemotherapy between 2006 and 2010. Eighty-six patients were men while 79 were women, and their average age was 57 years (range, 38-84 years). Clinicopathologic characteristics of these patients include gender, age, location, tumor size, histological differentiation, lymph node metastasis, nerve invasion, TNM stage and Ki-67 expression. Eight pairs of fresh PDAC and adjacent normal samples were frozen in the liquid nitrogen and maintained at −80°C until use for Western blot analysis. Approval for this study was obtained from patients' consents and the Ethics Committee of Affiliated Hospital of Nantong University.

2.2. Western blot

Tissues and harvested cells were immediately homogenized in a homogenization buffer (50 mmol/L Tris-Cl, pH 7.5, 1 mmol/L EDTA, 1% TritonX-100, 1% NP-40) supplemented with complete protease and phosphatase inhibitors (Roche Diagnostics, Mannheim, Germany) and then centrifuged at 13 000*g* for 30 minutes to collect the supernatant. Total protein concentrations were detected by Bio-Rad protein assay (Bio-Rad, Hercules, CA). Before gel electrophoresis, the supernatant was diluted with 2× sodium dodecyl sulfate loading buffer and boiled for 15 minutes. An equivalent amount of protein from each sample was separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then transferred to a polyvinylidene fluoride membrane (Millipore, Bedford, MA). After blocking with 5% non-fat milk in TBST (20 mmol/L Tris, 150 mmol/L NaCl, 0.05% Tween-20), the membranes were incubated overnight at room temperature with the following primary antibodies: DIXDC1 (1:500; Santa Cruz Biotechnology, Inc, Santa Cruz, CA), proliferating cell nuclear antigen (PCNA) (1:1000, Santa Cruz Biotechnology, Inc), cyclin D1 (1:500, Santa Cruz Biotechnology, Inc), and β-actin (1:500, Santa Cruz Biotechnology, Inc) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1000, Santa Cruz Biotechnology, Inc). After 3 washes, the membranes were incubated with horseradish peroxidase–linked anti-mouse or anti-rabbit IgG (1:5000; Pierce). The membranes were visualized using an enhanced chemiluminescent detection reaction (NEN Life Science Products, Boston, MA). Western blot analyses were used to examine the levels of protein. All target proteins were normalized to β-actin or GAPDH to determine the expression differences.

2.3. Immunohistochemistry

Immunohistochemistry was used to assess the clinical significance of DIXDC1 in PDAC progression. In brief, the PDAC tissue microarray were deparaffinized using a graded ethanol series and were processed in 10 mmol/L citrate buffer (pH 6.0) and heated to 121°C in an autoclave for 3 minutes to retrieve the antigen. Endogenous peroxidase activity was blocked by immersion in 3% hydrogen peroxide for 20