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Oncogenic Function of ATDC in Pancreatic Cancer through Wnt Pathway Activation and β-Catenin Stabilization

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SUMMARY

Pancreatic cancer is a deadly disease characterized by late diagnosis and resistance to therapy. Much progress has been made in defining gene defects in pancreatic cancer, but a full accounting of its molecular pathogenesis remains to be provided. Here, we show that expression of the ataxia-telangiectasia group D complementing gene (*ATDC*), also called *TRIM29*, is elevated in most invasive pancreatic cancers and pancreatic cancer precursor lesions. ATDC promoted cancer cell proliferation in vitro and enhanced tumor growth and metastasis in vivo. ATDC expression correlated with elevated β-catenin levels in pancreatic cancer, and β-catenin function was required for ATDC's oncogenic effects. ATDC was found to stabilize β-catenin via ATDC-induced effects on the Disheveled-2 protein, a negative regulator of glycogen synthase kinase 3β in the Wnt/β-catenin signaling pathway.

INTRODUCTION

Pancreatic cancer is a highly lethal disease that is often diagnosed in an advanced state. In fact, though it is the fourth most common cause of cancer death in the United States, resulting in 37,000 deaths per year (Jemal et al., 2007), it has the worst prognosis of any major malignancy (<5% 5-year survival rate). Recent advances in surgical and medical therapy have had only a modest impact on pancreatic cancer mortality. A major hallmark of pancreatic cancer is extensive local tumor invasion and early systemic dissemination. Pancreatic cancer is also notoriously resistant to chemotherapy and ionizing radiation.

To further understand the molecular pathogenesis of pancreatic cancer, genomic and proteomic profiling has been performed to identify differentially expressed genes and proteins that might represent novel therapeutic targets (Cao et al., 2004; Chen et al., 2005; Logsdon et al., 2003; Lowe et al., 2007). Using Affymetrix gene expression profiling, we previously found that pancreatic cancer cells overexpress the ataxia-telangiectasia group D complementing gene (*ATDC*) at an average

SIGNIFICANCE

Pancreatic cancer is an aggressive malignancy, and an improved understanding of the molecular mechanisms governing its highly aggressive behavior is needed for more effective treatment, early detection, and prevention. Defects in Wnt/β-catenin signaling are common in certain cancers, such as colorectal carcinoma, and recent evidence suggests that Wnt/β-catenin signaling may contribute to pancreatic cancer. In this report, we show that the ataxia-telangiectasia group D complementing gene (*ATDC*) is overexpressed in the majority of invasive pancreatic cancers and pancreatic cancer precursor lesions. ATDC contributes to pancreatic cancer via its ability to interact with and stabilize expression of Disheveled-2, with resultant stabilization of β-catenin. Besides highlighting ATDC as a potential therapeutic target in pancreatic cancer, our studies have defined a mechanism for activating Wnt/β-catenin signaling in cancer.

level 20-fold higher than epithelial cells from normal pancreas or chronic pancreatitis-derived tissues (Logsdon et al., 2003). *ATDC* was initially described in the hunt for the gene responsible for the genetic disorder ataxia-telangiectasia (AT) (Kapp et al., 1992). Both alleles of the *ATDC* gene in an AT patient-derived cell line were found to contain early stop codon mutations leading to a truncated and nonfunctional ATDC protein (Tauchi et al., 2000). No germline *ATDC* mutations were found in AT patients, and subsequently the gene responsible for AT was identified as ataxia-telangiectasia mutated (*ATM*) (Savitsky et al., 1995).

The *ATDC* gene, located at chromosome 11q23, encodes a 588 amino acid protein with multiple zinc-finger motifs and an adjacent leucine-zipper motif that may allow the ATDC protein to form homo- or heterodimers (Kapp et al., 1992). Northern blot analysis revealed that *ATDC* is normally expressed in placenta, lung, thymus, prostate, testis, and colon, while no expression is observed in heart, brain, skeletal muscle, pancreas, spleen, ovary, or small intestine (Hosoi and Kapp, 1994). In addition to our observation of high *ATDC* levels in primary pancreatic cancers and pancreatic cancer cell lines, a search of the Oncomine database (http://www.oncomine.org/) revealed that *ATDC* has been reported to be overexpressed in lung (Hawthorn et al., 2006), bladder (Dyrskjot et al., 2004), colorectal (Glebov et al., 2006; Ohmachi et al., 2006), ovarian (Santin et al., 2004), and endometrial cancers (Mutter et al., 2001) and in multiple myeloma (Zhan et al., 2002), with apparent reduced expression in melanoma (Smith et al., 2005) and in breast (Nacht et al., 1999), head and neck (Zhang et al., 2006), and prostate cancers (LaTulippe et al., 2002; Luo et al., 2001; Yu et al., 2004). A recent report identified a correlation between *ATDC* expression in gastric cancer and poor histological grade, large tumor size, extent of tumor invasion, and lymph node metastasis (Kosaka et al., 2007).

The ATDC protein, also known as TRIM29, is a member of the tripartite motif (TRIM) family. TRIM proteins have a series of conserved domains, which include a RING (R), a B box type 1 (B1) and B box type 2 (B2), and a coiled-coil (CC) region. While some of the domains may be absent or present in the different TRIM proteins (ATDC contains the B1-B2-CC domains but lacks the R domain), their order is always maintained (R-B1-B2-CC) (Reymond et al., 2001). Proteins belonging to the TRIM family have been implicated in a variety of cellular processes, such as development and growth, and in several human diseases, including HIV infection (Stremlau et al., 2004) and leukemia (Goddard et al., 1991). The function of ATDC has not been studied previously in any physiologic or pathologic process, though the ATDC protein appears to be localized primarily to the cytoplasm (Reymond et al., 2001). In this report, we describe our data examining the expression and functional role of ATDC in pancreatic cancer.

RESULTS

ATDC Is Overexpressed in Human Pancreatic Adenocarcinoma

To identify genes with potential roles in the development and progression of pancreatic adenocarcinoma, we assessed gene expression in microdissected samples of human pancreatic carcinoma using Affymetrix arrays. We compared the expression patterns in cancer tissues to those seen in normal pancreas and chronic pancreatitis, the latter of which served as an important control for the extensive fibrosis typically observed with pancreatic cancer (Logsdon et al., 2003). One of the most highly upregulated genes was the *ATDC* gene, which showed elevated expression in 10 out of 10 samples of pancreatic cancers relative to normal pancreas or chronic pancreatitis samples (Figure 1A). On average, *ATDC* expression was roughly 20-fold higher in pancreatic adenocarcinomas. The gene expression data were further confirmed in an analysis of *ATDC* mRNA levels of pancreatic cancer using quantitative real-time RT-PCR (qRT-PCR) (Figure 1B). Immunohistochemical staining confirmed that ATDC protein expression was present in the neoplastic epithelium of pancreatic cancer (Figure 1C).

A pancreatic cancer progression model is now widely accepted in which normal pancreatic ductal epithelium progresses to infiltrating cancer through a series of morphologically defined pancreatic precursors called pancreatic intraepithelial neoplasias (PanINs) (Hruban et al., 2000). This progression is associated with accumulation of specific genetic changes, such as K-ras mutations and inactivation of p16, that are observed in invasive pancreatic cancer. We found that ATDC was not expressed in PanIN 1 lesions (0 of 4) but was occasionally expressed in PanIN 2 lesions (1 of 7) and was more often expressed in PanIN 3 lesions (3 of 6) (Figure 1D). These data suggest that upregulation of ATDC occurs prior to the development of invasive pancreatic cancer.

ATDC Promotes Cellular Proliferation In Vitro and Pancreatic Tumorigenesis In Vivo

To understand the function of ATDC in pancreatic cancer, we first explored the effect of ectopic *ATDC* expression on cellular growth in vitro in multiple cell lines with differing levels of endogenous ATDC expression. Following transfection with an *ATDC* cDNA expression construct, HEK293 cells, which normally do not express ATDC, and MiaPaCa2 pancreatic cancer cells, which express low endogenous levels of ATDC, demonstrated a significant increase in cellular proliferation (Figures 2A and 2B). Similar changes were observed in monoclonal and polyclonal HEK293 cells lines stably overexpressing ATDC (see Figure S1 available online). Conversely, cellular proliferation was attenuated when endogenous ATDC expression was silenced by stable transfection with two different shRNA vectors targeting distinct regions of *ATDC* in Panc1 and BxPC3 pancreatic cancer cell lines, both of which have high endogenous levels of ATDC (Figures 2C and 2D). Expression of ATDC shRNAs 1 and 2 did not alter basal cell proliferation rates in HEK293 cells (Figure S2), verifying the specificity of the inhibitory function of the ATDC shRNAs on ATDC's function.

To examine the effects of ATDC silencing on pancreatic tumor growth in vivo, we infected Panc1 cells expressing a control shRNA or ATDC shRNA1 with a luciferase-expressing lentivirus. Following injection of 5×10^5 cells into the tail of the pancreas, tumor growth was assessed using bioluminescent imaging (n = 8 animals per group). All of the animals injected with Panc1 cells expressing control shRNA demonstrated tumor formation 14 days postinjection, while tumors were not detected in the animals injected with Panc1 ATDC shRNA cells (Figure 2E, left panels). At 60 days postinjection, the control shRNA animals' tumors grew significantly larger, with evidence of metastatic