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Expression of Coiled-Coil Domain Containing 34 (CCDC34) and its Prognostic Significance in Pancreatic Adenocarcinoma

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Coiled-coil domain containing 34 (CCDC34) promotes cell proliferation and invasive properties in human cancer. The aim of this study was to compare the expression of CCDC34 in pancreatic adenocarcinoma with normal pancreatic tissue, and to evaluate the prognostic significance of CCDC34 expression in patients with pancreatic adenocarcinoma, using bioinformatics.

Material/Methods: The expression and prognostic value of CCDC34 were initially predicted using Oncomine and The Cancer Genome Atlas (TCGA) databases. Pancreatic adenocarcinoma tissue samples (N=90) and matched normal pancreatic tissues (N=90) were studied using immunohistochemistry to measure CCDC34 protein expression levels. Univariate Kaplan-Meier, and multivariate Cox analysis were used to determine the prognostic role of CCDC34 expression.

Results: Oncomine and TCGA databases predicted that CCDC34 mRNA expression levels were significantly increased in pancreatic adenocarcinoma compared with normal pancreatic tissues ($P<0.05$), and that patients with increased CCDC34 mRNA expression levels had significantly lower overall survival (OS) ($P=0.031$). Immunohistochemistry showed that expression levels of CCDC34 protein in pancreatic adenocarcinoma were significantly increased, compared with normal pancreas ($P=0.000$). Patients with pancreatic adenocarcinoma with increased expression of tissue CCDC34 had significantly reduced OS compared with patients with low expression ($P=0.000$). Univariate and multivariate survival analysis showed that increased expression of CCDC34 was an independent predictor of poor prognosis in patients with pancreatic adenocarcinoma (all, $P=0.000$).

Conclusions: Compared with normal pancreas, CCDC34 expression was significantly increased in pancreatic adenocarcinoma, and increased CCDC34 expression was an independent predictor of poor patient prognosis.

MeSH Keywords: Pancreatic Neoplasms • Prognosis • rho-Associated Kinases

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Background

Pancreatic adenocarcinoma is one of the most common malignant tumors of the digestive tract [1]. Pancreatic adenocarcinoma has the characteristics of early invasion and metastasis, with a low rate of detection in the early stage [2]. Although diagnosis and treatment strategies for patients with pancreatic adenocarcinoma have improved in recent decades, the prognosis for patients remains poor, even following radical surgery [3]. Therefore the identification of novel therapeutic targets and prognostic biomarkers are urgently needed to improve the prognosis for patients with pancreatic adenocarcinoma.

Coiled-coil domain containing 34 (CCDC34) is a protein-coding and disease-related gene, which is also known as NY-REN-41, RAMA3 or L15 [4]. CCDC34 contains 373 amino acids and is located on chromosome 11p14.1 [4]. The chromosomal abnormality associated with CCDC34, translocation t(11;18) (p13;p11.2) was first observed in a patient with a hamartoma of the retinal pigment epithelium and retina [4]. Subsequently, CCDC34 has been found to be overexpressed in several human malignancies, including renal-cell carcinoma [5], non-small-cell lung cancer (NSCLC) [6] and bladder carcinoma [7]. However, the expression of CCDC34 in pancreatic adenocarcinoma, and its clinical significance, have not been previously reported.

Therefore, the aim of this study was to compare the expression of CCDC34 in pancreatic adenocarcinoma with normal pancreatic tissue, and to evaluate the prognostic significance of CCDC34 expression in patients with pancreatic adenocarcinoma, using bioinformatics prediction combined with immunohistochemical validation.

Material and Methods

Bioinformatics prediction

The cancer-related public databases, Oncomine and The Cancer Genome Atlas (TCGA) were searched for predictive bioinformatics data. In the Oncomine database, the search term, 'CCDC34' was used and the differential gene analysis module was chosen (pancreatic cancer *vs.* normal) to retrieve the results. Pancreatic adenocarcinoma-related data from TCGA database was downloaded, and included a total of 179 cases of patients with pancreatic adenocarcinoma and 171 cases of normal pancreas with CCDC34 gene expression information. According to the different expression levels (high *vs.* low), the survival curve was plotted to include the relationship between CCDC34 mRNA expression and patient overall survival (OS) curves were drawn by the Kaplan-Meier method.

Pancreatic adenocarcinoma specimens and clinicopathological data

A total of 90 cases of tumor tissue samples and paired adjacent normal tissues were retrospectively collected from patients with pancreatic adenocarcinoma, who had received radical surgery from September 2004–December 2008 in our hospital. Patient-related clinical information, including gender, age, tumor location, TNM stage, are shown in Table 1. This study was approved by the Ethics Committee of Anhui Provincial Hospital. All patients signed an informed consent to participate in the study.

Immunohistochemical staining and interpretation of the results

Immunohistochemical staining was performed according to the manufacturer's protocol, using the primary antibody to CCDC34 antibody (ab122396) (Abcam, Cambridge, UK) at a dilution of 1: 500. The results were determined by light microscopy, with the immunostaining of each tissue section independently assessed by two histopathologists, as previously reported [8]. A visual immunoreactive score (IRS) was used for each case, ranging from 0–12, which was generated by staining intensity (SI) × number of stained cells (PP). The detailed scoring criteria for the SI (score, 0–3): 0 was negative, 1 was weakly positive, 2 was moderately positive and 3 was strongly positive. The detailed scoring criteria for the PP (score, 0–4): 0 was negative, 1 was 1–25%, 2 was 26–50%, 3 was 51–75%, 4 was 76–100%. If the IRS score was >4, the CCDC34 expression was defined as high; and if the IRS score was ≤4, the CCDC34 expression was defined as high.

Statistical analysis

SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA) was used to analyze the experimental data. Quantitative data were expressed as the mean ± standard deviation (SD). The correlation between CCDC34 expression and clinicopathological parameters was analyzed by the Chi-squared test. Survival analysis was calculated by the Kaplan-Meier method, and by the log-rank test. The Cox regression model was established for multivariate survival analysis to determine prognostic factors that were significant in the univariate analysis. A P-value <0.05 was considered to be statistically significant.

Results

Overexpression of CCDC34 mRNA and protein levels in pancreatic adenocarcinoma

Oncomine and The Cancer Genome Atlas (TCGA) databases were used to predict the CCDC34 mRNA expression levels in

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Table 1. Correlation of CCDC34 with clinicopathological parameters of patients with pancreatic adenocarcinoma.

Variables	Cases (N)	CCDC34 expression		*P* value
		Low	High	
Age at surgery (years)				
≤60	42	12	30	0.875
>60	48	13	35	
Gender				
Male	57	14	43	0.371
Female	33	11	22	
Tumor location				
Head	51	16	35	0.384
Body/tail	39	9	30	
Tumor diameter (cm)				
≤2	76	20	56	0.471
>2	14	5	9	
Histological grade				
Well	60	18	42	0.506
Moderate/poor	30	7	23	
Neural/vascular invasion				
Yes	39	7	32	0.069
No	51	18	33	
Lymph node metastasis				
Yes	33	8	25	0.569
No	57	17	40	
TNM stage				
I–IIA	52	17	35	0.223
IIB–IV	38	8	30	

patients with pancreatic adenocarcinoma and normal pancreatic tissues. Compared with the normal group, the expression level of CCDC34 mRNA was significantly greater in tissue sections of pancreatic adenocarcinoma (all values, P<0.05) (Figure 1A–1C). To verify the above predictions, an immunohistochemical method was used in 90 cases of pancreatic adenocarcinoma and paired adjacent normal pancreatic tissues (Figure 2A, 2B). The results showed that the CCDC34 protein was mainly expressed in the cell membrane and cytoplasm of the pancreatic adenocarcinoma cell (Figure 2A). The expression level of CCDC34 protein in pancreatic adenocarcinoma tissues was significantly greater compared with the paired normal pancreatic tissue samples (P=0.000) (Table 2).

Relationship between CCDC34 differential expression and clinicopathological parameters of patients with pancreatic adenocarcinoma

The relationship between CCDC34 protein expression and clinicopathological parameters of the patients with pancreatic adenocarcinoma were studied, including age, gender, tumor location, tumor diameter, histological grade, neural/vascular invasion, lymph node metastasis, and TNM stage. As shown in Table 1, there were no significant correlations between the CCDC34 protein expression levels and the clinicopathological parameters in patients with pancreatic adenocarcinoma.

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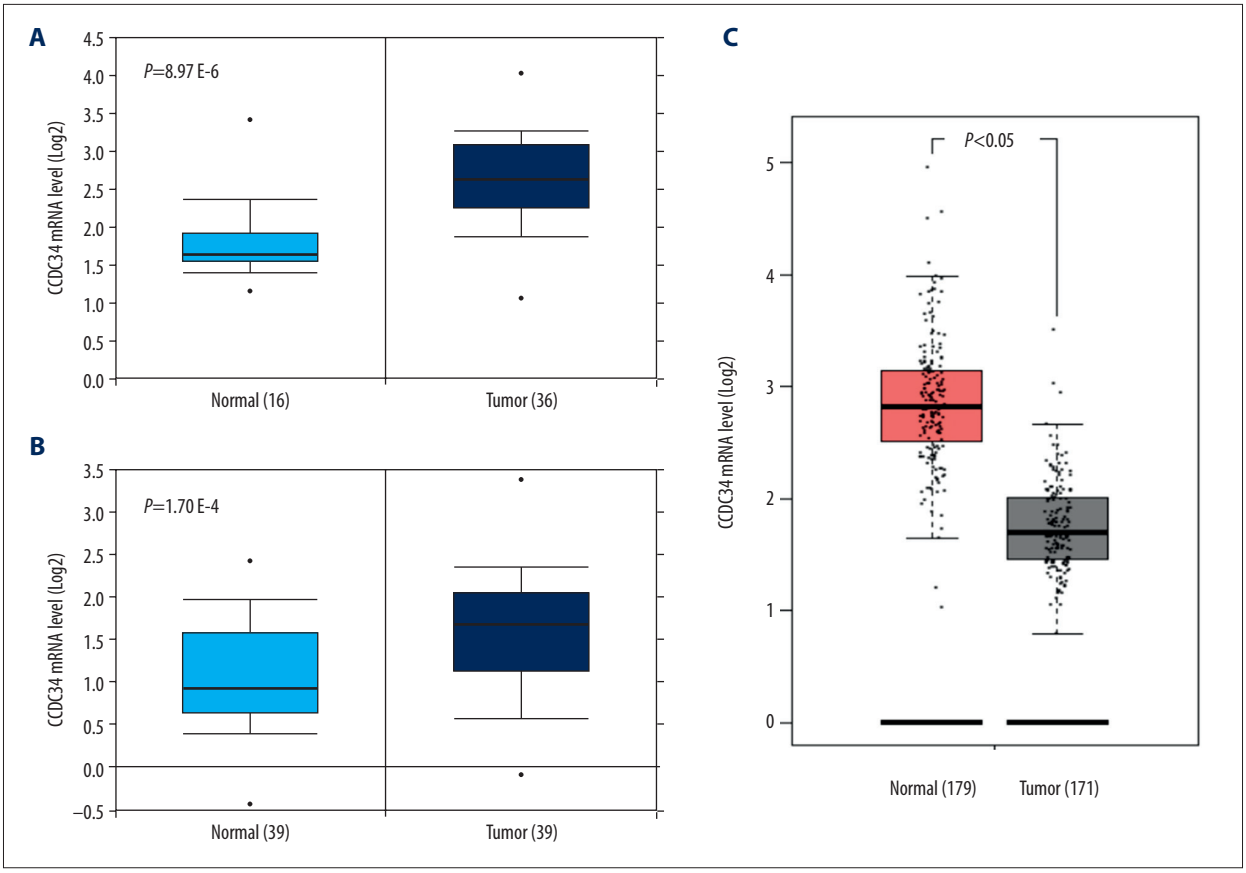

Figure 1. Increased expression of coiled-coil domain containing 34 (CCDC34) mRNA in pancreatic adenocarcinoma predicted from the Oncomine and The Cancer Genome Atlas (TCGA) databases grouped by pancreatic cancer *vs.* normal pancreas Coiled-coil domain containing 34 (CCDC34) mRNA levels in: (**A**) Pei Pancreas (GEO: GSE 16515); (**B**) Badea Pancreas (GEO: GSE 15471); (**C**) TCGA database.

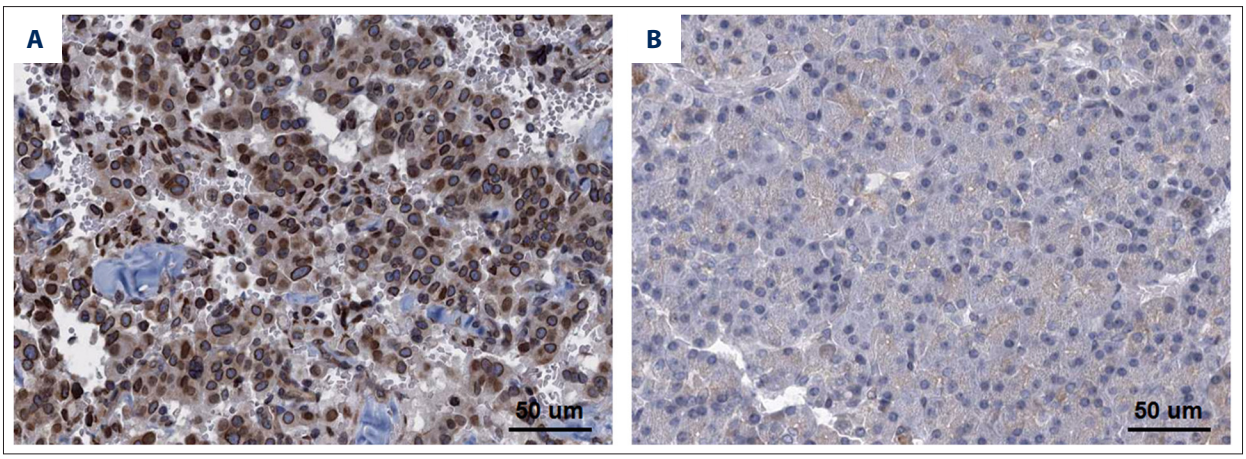

Figure 2. Representative photomicrographs of the immunohistochemical staining for coiled-coil domain containing 34 (CCDC34) in 90 cases of paired pancreatic adenocarcinoma and adjacent normal pancreatic tissues. (**A**) Increased expression of CCDC34 in pancreatic adenocarcinoma tissue. (**B**) Low expression of CCDC34 in matched adjacent normal pancreatic tissue. Bar=50 mm.

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Table 2. High expression of CCDC34 in 90 cases of pancreatic adenocarcinoma compared to the paired adjacent normal tissues.

Samples	CCDC34 expression levels (cases, %)		*P*
	Low	High	
Pancreatic adenocarcinoma	25 (27.8)	65 (72.2)	0.000
Normal tissues	51 (56.7)	39 (43.3)	

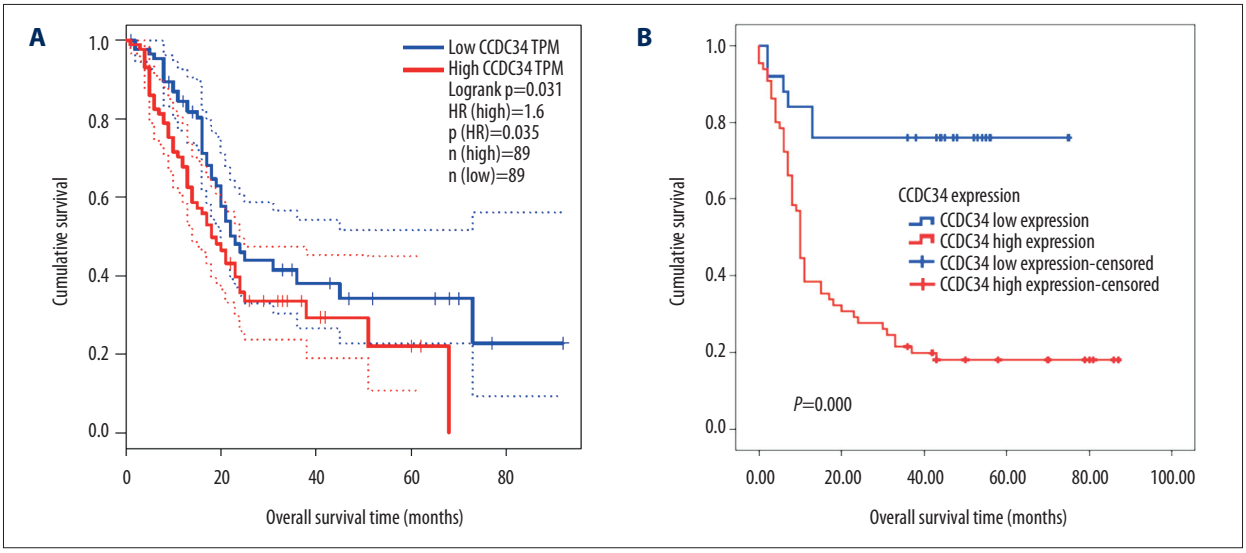

Figure 3. Kaplan-Meier curves of overall survival (OS) of patients with pancreatic adenocarcinoma based on expression of coiled-coil domain containing 34 (CCDC34) (high versus low). (**A**) The relationship between CCDC34 mRNA expression level and overall survival (OS) of patients with pancreatic adenocarcinoma based on The Cancer Genome Atlas (TCGA) database. (**B**) The relationship between CCDC34 protein expression levels and OS of patients with pancreatic adenocarcinoma based on the immunohistochemical data.

Prognostic value of CCDC34 expression in patients with pancreatic adenocarcinoma

The relationship between the differential expression of CCDC34 and the prognosis of patients with pancreatic adenocarcinoma was studied. The results from the TCGA database showed that the overall survival (OS) of patients with pancreatic adenocarcinoma with high expression of CCDC34 mRNA was significantly lower than that of patients with low expression of CCDC34 mRNA (P=0.031) (Figure 3A). To verify this result, a statistical analysis of the results of immunohistochemistry with bioinformatics data found that the OS of patients with pancreatic adenocarcinoma with high expression of CCDC34 protein was significantly less than that of patients with low expression (P=0.000) (Figure 3B). As shown in Table 3, Kaplan-Meier univariate survival analysis showed that the expression of CCDC34 (high expression *vs.* low expression), histological grade (well *vs.* moderate/poor), lymph node metastasis (present *vs.* not present), and TNM stage (stage I–IIA versus IIB–IV) were the significant factors influencing the survival time of patients with pancreatic adenocarcinoma. These four significant single factors were substituted into the Cox multivariate survival analysis, and the results showed that the high expression level of CCDC34 and histological grade were the independent factors that were predictive for unfavorable prognosis in patients with pancreatic adenocarcinoma (P=0.000 and 0.007, respectively) (Table 4).

Discussion

Coiled-coil is a prevalent protein domain, and proteins with a coiled-coil structure are structural proteins, membrane proteins, enzymes, and transcription factors [9]. The spatial folding of coiled-coil domain-containing (CCDC) is variable, resulting in different spatial conformations, achieving many different molecular biological functions, including regulating gene expression, cell division, membrane fusion and drug controlled release [10]. It has previously been confirmed that the CCDC gene structure or the epigenetic changes associated with many malignant tumors and aberrant expression CCDC related protein have been demonstrated in nasopharyngeal carcinoma [11], breast cancer [12,13], non-small cell lung cancer (NSCLC) [14,15], gastric cancer [16,17], colorectal cancer [18]

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Table 3. Kaplan-Meier survival analysis of CCDC34 and other clinicopathological parameters in patients with pancreatic adenocarcinoma.

Variables	Mean survival time (months)	95% CI	*P* value
CCDC34 expression			
Low	58.720	47.330–70.110	0.000
High	25.099	17.658–32.541	
Age at surgery (years)			
≤60	37.363	26.492–48.233	0.751
>60	36.247	25.666–46.828	
Gender			
Male	30.871	21.931–39.812	0.053
Female	41.641	30.409–52.873	
Tumor location			
Head	37.954	27.665–48.243	0.743
Body/tail	35.128	23.958–46.299	
Tumor diameter (cm)			
≤2	37.686	29.327–46.046	0.456
>2	29.643	13.698–45.588	
Histological grade			
Well	42.429	33.023–51.836	0.017
Moderate/poor	24.233	13.110–35.357	
Neural/vascular invasion			
Yes	29.570	19.775–39.365	0.276
No	41.354	30.731–51.977	
Lymph node metastasis			
Yes	25.384	14.487–36.280	0.029
No	43.432	33.591–53.274	
TNM stage			
I–IIA	46.308	35.897–56.718	0.004
IIB–IV	24.013	14.346–33.680	

Table 4. Cox multivariate analysis of CCDC34 and other clinicopathological parameters in patients with pancreatic adenocarcinoma.

Covariates	HR	95% CI for HR	*P* value
CCDC34 expression (low *vs.* high)	5.461	2.319–12.864	0.000
Histological grade (well *vs.* moderate/poor)	2.146	1.228–3.752	0.007
Lymph node metastasis (No *vs.* Yes)	1.457	0.556–3.814	0.444
TNM stage (I–IIA *vs.* IIB–IV)	1.548	0.612–3.915	0.356

and hepatocellular carcinoma [19,20]. Recently, CCDC34, as a new member of CCDC related protein family, has been shown to be overexpressed in bladder carcinoma, and knockdown of its expression has been shown to inhibit the invasion and migration of tumor cells [7]. However, the expression of CCDC34 in pancreatic adenocarcinoma and its prognostic significance has remained unclear.

For these reasons, this study used bioinformatics to predict the high expression of CCDC34 in pancreatic adenocarcinoma

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tissues by Oncomine and The Cancer Genome Atlas (TCGA) public databases. Immunohistochemical detection of CCDC34 protein expression was used to verify that CCDC34 expression was significantly increased in pancreatic adenocarcinoma compared with the normal control group and showed that the difference was statistically significant. These data results were consistent with bioinformatics predictions and also suggest that CCDC34 may play a role in promoting the development of pancreatic adenocarcinoma by acting as an oncogene.

The clinical prognostic significance of CCDC34 expression levels in patients with pancreatic adenocarcinoma was investigated using Kaplan-Meier univariate survival analysis, which showed that patients with pancreatic adenocarcinoma with increased expression of CCDC34 mRNA and protein levels reduced overall survival (OS) compared with low levels of CCDC34. Cox multivariate analysis showed that the high expression of CCDC34 was an independent predictive for unfavorable prognosis in patients with pancreatic adenocarcinoma. These findings indicated that high levels of expression of CCDC34 could indicate poor prognosis for patients with pancreatic adenocarcinoma, which may be a key target gene involved in the process of pancreatic adenocarcinoma cell growth and metastasis.

This study had several limitations. The cases were collected non-sequentially, which may have resulted in selection bias and influenced the findings. Also, some clinicopathological data associated with pancreatic adenocarcinoma were not included, which may have had an impact on the overall analysis of the results. Also, the detailed underlying molecular mechanisms associated with CCDC34 expression and pancreatic adenocarcinoma await further investigation.

Conclusions

The findings of this study showed that the overexpression of CCDC34 was an independent predictor of reduced prognosis of patients with pancreatic adenocarcinoma. The findings of this study support the need for further studies on CCDC34 expression as a possible prognostic marker for pancreatic adenocarcinoma or as a therapeutic target.

Conflicts of interest

None.

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