Expression and clinical significance of P120 catenin mRNA and protein in pancreatic carcinoma

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Abstract

The aim of this work was investigate the association of P120 catenin expression with the clinicopathologic features and prognosis of pancreatic carcinoma. RT-PCR was performed to investigate the expression of P120 catenin mRNA and western blotting were performed to investigate the expression of P120 catenin protein in 52 patients with pancreatic carcinoma. The relationships between P120 catenin expression and clinicopathological characteristics and prognosis were analyzed.

The mRNA and protein expression of P120 catenin detected by RT-PCR and western blotting in pancreatic carcinoma was significantly lower than that in normal pancreatic tissues (0.227±0.067 vs 0.793±0.162, t=9.157, P =0.000; 0.665±0.192 vs 0.936±0.251, t=3.857, P=0.002). Reduced expression of P120 catenin mRNA and protein was significantly correlated with lymph node metastasis (P =0.004, P =0.006), vascular invasion (P =0.022, P =0.039), distant metastasis (P =0.037 , P =0.025), differentiated (P =0.033, P =0.013) and pTNM stage (P =0.003, P =0.022) of tumours. Additionally, reduced expression of P120 catenin mRNA and protein in tumour correlated with a worse prognosis and normal expression with a better survival rate (P=0.022, P=0.007). The reduced expression of both P120 catenin mRNA and protein in pancreatic carcinoma suggest that low expressions relate to pancreatic carcinoma development. P120 catenin may be related to pancreatic carcinoma behaviour and be a potential prognostic molecule.

KEY WORDS: P120 catenin, RT-PCR, western blotting, pancreatic carcinoma.

Introduction

Pancreatic carcinoma is one of the most frequent causes of cancer-related death worldwide (1). As it has aggressive behaviour and high metastatic potential, the mortality rate of patients with pancreatic carcinoma is high. Moreover, it is not responsive to chemotherapy or radiotherapy, and so the overall 5-year survival rate is less than 5% with median survival after diagnosis being approximately six months (2). The identification of a sensitive and representative disease marker is important for prognostication and as a guide for appropriate treatment. P120 catenin (P120ctn) was first identified as a prominent tyrosine kinase substrate for Src, but has been also identified to become tyrosine phosphorylated in response to receptor signaling through platelet-derived growth factor, colony-simulating factor-1, and epidermal growth factor (3). P120ctn binds directly to cytoplasmic domain of E-cadherin which plays a key role in cell-cell interaction by armadillo1-7 repeat domain (Arm domain), subsequently both of them constitute cadherin-catenin complex(CCC)(4). It regulates E-cadherin turnover on the cell surface and its dissociation promotes E-cadherin internalization. It also stabilizes E-cadherin and rescues its function posttranslationally (5). Besides cellular adhesive function, P120ctn is also involved in the regulation of cell motility through actin cytoskeleton remodeling via Rho, Rac and Cdc42 GTPases (6). Recently, altered expression and down-regulation of P120ctn was reported to correlate with occurrence, development and prognosis of various cancers (7-9). To date, however, there is no clinical trial verifying the relationship between P120ctn and pancreatic carcinoma. In this study, expression of P120ctn was investigated in pancreatic carcinoma tissues and control tissues using reverse transcriptase-polymerase chain reaction (RT-PCR) and western blotting. And the relationship of P120ctn expression to the clinicopathological features and patient survival of pancreatic carcinoma was investigated.

Materials and Methods

Patients and tissue specimens

Excisional paired specimens of pancreatic carcinoma tissues and normal pancreas tissues were obtained from the department of General Surgery, Zhongda Hospital affiliated Southeast University between January 2001 and January 2006. Tumour specimens and paired normal pancreas tissues were obtained immediately after surgical resection, the normal pancreas tissues were taken more than 20mm away from the pancreatic carcinoma. Half of each tissue sample was immediately fixed in 10% buffered formalin (Tianlong, Nanjing, China) and embedded in paraffin. Section (4μm thick) were prepared for haematoxylin and eosin (H.E.) (Dingtian, Shanghai, China) staining for histological diagnosis. The other half of the tissue was shape-frozen in liquid nitrogen and stored at -70°C until used for RT-PCR or western blotting.

Clinicopathological Data

Detailed Clinicopathological characteristics including gender, age, serum carcinoembryonic antigen (CEA) and carbohydrate antigen199 (CA199), tumour size, degree of differentiation, location of tumour, depth of invasion, vascular invasion, lymph node metastasis, distant metastasis and pTNM stage of patients were recorded (Table 1). Staged according to the 2002 tumour node metastasis (TNM) classification recommended by the international Union Against Cancer TNM classification (UICC). The study patients consisted of 52 patients in four TNM stages: stage I (n=17), stage II (n=12), stage III (n=15) and stage IV (n=8).

RT-PCR

Total RNA from specimens analyzed for P120 catenin expression was extracted using a commercial Kit (Gibco BRL, Paisley, UK). Total RNA (0.5μg) was reverse-transcribed using the M-MLV Reverse Transcriptase (Zhongshan, Beijing, China) with incubation of 2 h at 37 °C, followed by 5 min at 95°C ,and diluted to 1:5 with RNase-free water. RT-PCR was performed on complementary DNA samples using a DNA thermal cycler (Eppendorf Mastercycler gradient, Hamburg, Germany) with Reddymix PCR master mix (ABgene, Surrey, UK). DJ primers were constructed using the gene sequence obtained by BLAST search in the primer3 output program. Primer sequence was as follows: human P120ctn (ACCESSION AF062344), Sense 5' -TCGGGAATGTGATGGTTT-3', antisense 5'-GCTTCTAGGATGGCAGGA-3' (product size, 273bp). β-actin Sense 5'-GGGACCTGACTGACTACCTC-3', Antisense 5'-CGTCATACTCCTGCTTGCTG-3' (product size, 406bp). The primer sequences were synthesized by Sangon Bioengineering Company (Shanghai, China). Conditions were as follow: P120ctn-heating at 95 °C for 5 min, denaturation at 94°C for 30 sec, annealing at 53 °C for 30 sec, extension at 72 °C for 40 sec, for 32 cycles; β-actin-heating at 95 °C for 5 min, denaturation at 94 °C for 30 sec, annealing at 57°C for 30 s, extension at 72 °C for 40 s, for 30 cycles; finial-extension at 72 °C for 8 min all of them. Products were separated on 1.5% agarose gels, isolated using the Invisorb Spin DNA extraction kit (Invitek GmbH, Berlin, Germany) and sequenced. Gels were photographed by the Kodak EDAS 290 system (Kodak,

NY, USA). Densitometric analysis of films was performed using a computerized image analysis (NIH IMAGE 1.63) program.P120ctn mRNA levels were established by calculating the target molecule/β-actin ratio (all cases scored for band intensity compared with control).

Western blotting

Pancreas tissues lysed with preparation of modified radioimmunoprecipitation buffer. Specimens were washed twice in TBS (20 mmol/L Tris-HCl, 500 mmol/L NaCl, 0.05% Tween-20) (10 min each time) and after centrifugation at 12000g for 10 min, the supernatant was extracted in bicinchoninic acid protein assay (Rockford, IL, USA). Thirty micrograms of protein sample was loaded onto a 10% SDS-PAGE and electroblotted onto a PVDF nylon membrane (Millipore, MA, USA). Membranes were blocked in TBS containing 5% skim milk, and then incubated with rabbit monoclonal P120ctn antibodies (1:200 dilution, No.P1870-200UL, 053K4812, Sigma, USA) and rabbit polyclonal β-actin antibodies (Santa Cruz, CA, USA)at 4°C overnight. Membranes were then incubated with a HRP-link goat anti-rabbit IgG secondary antibody (1:5000 dilution; Santa Cruz, CA, USA) at room temperature for 1 h. Finally, immunoteactive proteins were visualized on autoradipgram using ELC western blotting detection reagents (Amershan Pharmacia, Uppsala, Sweden) and exposed to X-Omat BT film (Kodak, NY, USA).Bands were analyzed by Quantity One Analyses Software normalized with respect to β-actin as an internal control.

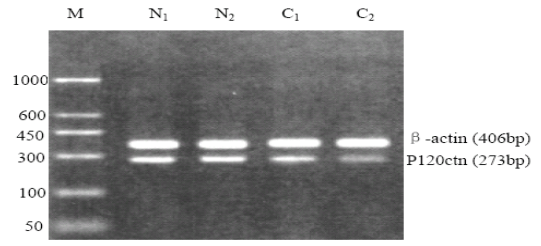
Statistical analysis

Statistical analyses were performed with SPSS software (Statistical Package for the Social Sciences, version 13.0, SSPS, Inc., Chicago, IL). Continuous data were expressed as the mean+SD. The Student t-test and one-way analysis of variance (ANOVA) were used for continuous variables. For survival assessment, the Kaplan–Meier method with log-rank analysis was used. A *P*-value less than 0.05 was accepted as significant for all calculations.

RESULTS

Expression of P120ctn mRNA in paired specimens of pancreatic carcinoma

Using reverse transcriptase-polymerase chain reaction analysis, we analysis the mRNA expression of P120ctn. 1.5% agarose gels electrophoresis showed a 273bp P120ctn fragment from pancreatic carcinoma and normal pancreas specimens (Figure 1). The P120ctn mRNA amplification were seen in all tissues. The transcript levels were 0.227±0.067 in tumour significantly lower than that in normal pancreas tissues (0.793±0.162, t=9.157, P =0.000).



Lane M DNA marker, Lane C1,C2, pancreatic carcinoma tissues, Lane N1,N2, normal pancreas tissues

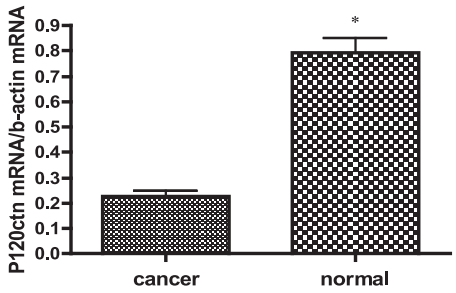


FIGURE 1. Expression of P120ctn mRNA in pancreatic cancer tissues and normal pancreas tissues (*P<0.05, carcinoma groups VS normal tissues groups)

Expression of P120ctn protein in paired specimens of pancreatic carcinoma

The affinity-purified anti-P120ctn antibody detected a major band at 120ku in protein extracts from all specimens tested (Figure 2.), The expressions

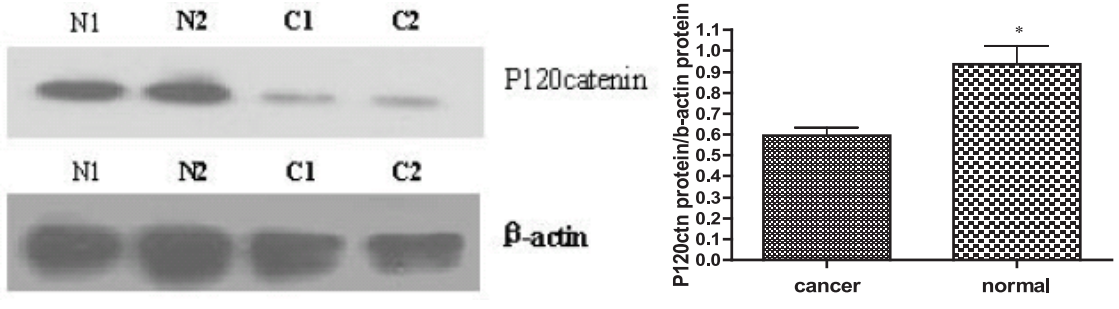


FIGURE 2. P120ctn protein expression in pancreatic cancer tissues and normal pancreas tissues (*P<0.05, carcinoma groups VS normal tissues groups)

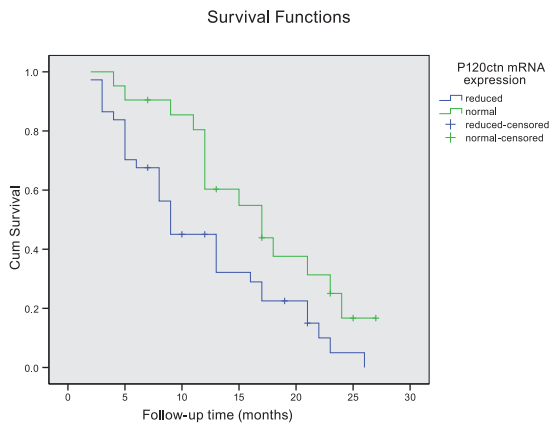


FIGURE 3. Kaplan-Meier Survival curves of patients with pancreatic cancer according to the expression of P120ctn mRNA

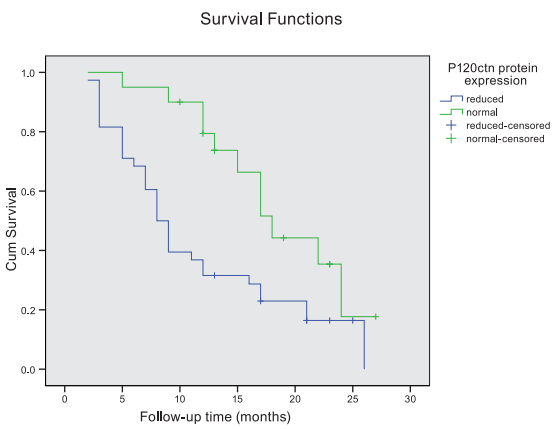


FIGURE 4. Kaplan-Meier Survival curves of patients with pancreatic cancer according to the expression of P120ctn protein.

were 0.665±0.192 in tumour much lower than that in normal pancreas tissues (0.936±0.251, t=3.857, P=0.002) which was consistent with that of RT-PCR.

Relationship between expression of P120ctn protein and clinicopathological characteristics of pancreatic carcinoma. Relationship between expression of P120ctn and clinicopathological characteristics of pancreatic carcinoma

characteristics	cases	mRNA	*t*-value	*P*-value	protein	*t*-value	*P*-value
Gender			0.493	0.639		0.075	0.942
Male	31	0.216±0.064			0.664±0.192		
Female	21	0.204±0.066			0.672±0.185		
Age			0.853	0.422		0.546	0.596
≤ 60years	24	0.222±0.061			0.645±0.160		
>60years	28	0.201±0.061			0.687±0.168		
CEA			1.041	0.328		0.887	0.391
≤5μg/L	19	0.222±0.059			0.659±0.175		
>5μg/L	33	0.203±0.057			0.609±0.116		
CA199			0.967	0.357		1.099	0.289
≤29KU/L	16	0.219±0.057			0.661±0.172		
>29KU/L	36	0.203±0.054			0.601±0.119		
Tumour size			1.446	0.179		0.989	0.336
≤ 2cm	23	0.228±0.061			0.662±0.162		
> 2cm	29	0.201±0.051			0.613±0.118		
Degree of differentiation			3.786*	0.033		4.688*	0.013
Well-differentiated	17	0.237±0.066			0.729±0.159		
Moderately-differentiated	19	0.192±0.056			0.658±0.100		
Poorly-differentiated	16	0.187±0.062			0.649±0.091		
Location of tumour			0.759	0.464		0.382	0.708
Head of pancreas	37	0.210±0.059			0.629±0.122		
Body and tail of pancreas	15	0.225±0.058			0.650±0.171		
Vascular invasion			2.513	0.022		2.169	0.039
Negative	28	0.248±0.074			0.727±0.177		
Positive	24	0.189±0.056			0.640±0.100		
Lymph node metastasis			3.251	0.004		2.984	0.006
Present	22	0.186±0.052			0.634±0.094		
Absent	30	0.252±0.069			0.749±0.181		
pTNM stage			5.205*	0.003		3.371*	0.022
I stage	17	0.222±0.065			0.719±0.140		
II stage	12	0.186±0.054			0.670±0.098		
III stage	15	0.165±0.038			0.660±0.093		
IV stage	8	0.159±0.051			0.649±0.087		

TABLE 1. Relationship between expression of P120ctn and clinicopathological characteristics
mRNA means relative amount of mRNA(P120ctn mRNA/β-actin mRNA),
protein means relative amount of protein (P120ctn protein/β-actin protein)
* is *F*-value

The correlation of P120ctn mRNA and protein expression of P120ctn protein with clinicopathological parameters was showed in Table1. Statistical analysis showed the reduced expression of P120ctn was significantly correlated with lymph node metastasis (P =0.004 and P =0.006), vascular invasion (P =0.022 and P =0.039) and distant metastasis (P =0.037 and P =0.025).The expression of P120ctn tended to be reduced in poorly-differentiated tumours compared with moderately- and well -differentiated tumours (P =0.033 and P =0.013). In addition, the expression of P120ctn was inversely associated with pTNM stage of tumours (P =0.003 and P =0.022). But the correlation between the altered expression of P120ctn and gender, age, serum CEA level, serum CA199 level, tumour size, location of tumour was not statistically significant.

Relationship between P120ctn expression and patient survival

Survival was assessed on the P120ctn mRNA level and P120ctn protein level separately by Kaplan–Meier analysis. When the value of P120ctn/β-actin was lower than the mean, it was regarded as "reduced", others was regarded as "normal". patients had longer survivals compared with P120ctn reduced patients (log-rank test, t=5.229, P=0.022) (Figure 3). There is a similar result on protein level. (log-rank test, t=7.360, P=0.007) (Figure 4.).

Discussion

The P120ctn belongs to a conserved family of armadillo-related proteins, many of which are associated with adherence junctions (β-catenin, plakoglobin). The human P120ctn gene (CTNND1) is located in chromosomes 11q11 and consists of 21 exons (10-11). Yanagisawa and Anastasiadis reported that P120ctn joined in the course of cell motility and invasiveness which is important to the occurrence, and development of tumours, and P120ctn may behave as a tumour suppressor (12). Sarrio et al previously analyzed 86 breast carcinomas with respect to P120ctn alterations and found absent or reduced P120ctn expression in tumours (13). In other studies, down-regulated expression of P120ctn were also found in colon carcinoma, prostate carcinoma, esophageal squamous cell carcinoma, and lung carcinoma (7-9,14). In our study, we demonstrate that compared with the expression of P120ctn in normal pancreatic tissues, it was significantly lowered or even was not found in tumour tissues on the mRNA level. This is similar to results found on protein level. P120ctn expression is down-regulated by genetic mechanisms in cancer. P120ctn expression levels act as a master regulator or rheostat for overall E-cadherin which called a metastasis-suppressor gene (15). Cadherins comprise a superfamily of transmembrane cell-cell adhesion receptors involved in a variety of biological processes including development, morphogenesis, and tumour metastasis. Cadherins on adjacent cells bind to one another through their extracellular domains. The intracellular domains mediate a re-organization of the actin cytoskeleton and promote intracellular signalling through interaction with the catenins (16-18). P120ctn interacts directly with the so-called juxtamembrane domain (JMD) and play significant signaling roles in the nucleus, regulating cell proliferation and invasiveness (19). As we all know, there are lots of growth factor receptor (GFR) including epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), transforming growth factor receptor-α (TGFR-α) in tumours. After being combined with the ligand, GFR enhance the degree of phosphorylation, and accelerate the phosphorylation of P120ctn (20). It may available to the reduction of P120ctn expression on cellular membrane and cell junctions, consequently results in down-regulation of cell adhesion, up-regulation of cell migration. All of these may accelerate the invasion and metastasis of tumour. In previous study, we have reported that abnormal expression of P120ctn was significantly associated with expression of E-catering and VEGF in pancreatic carcinoma and three of them played an important role in the Wnt pathway which has been proved as a key fact of tumour aggressiveness (21). So we have reason to say reduced expression of P120ctn may induce the occurrence and development of pancreatic carcinoma. Besides the mechanism mediating the reduced P120ctn expression, we found significant association between its expression and clinicopathological parameters or survival. In our study, P120ctn reduced expression in pancreatic carcinoma are significant associated with tumour differentiation, lymph node metastasis, vascular invasion, distant metastasis and pTNM stage of tumours, indicating a correlation with biological aggressiveness. Moreover, P120ctn expression was significantly associated with patient survival, patients with reduced expression of P120ctn were more likely to have disease progression or to die than those with normal expression of P120ctn. These data were in agreement with previous findings demonstrating an altered expression of P120ctn in tumours of breast, lung and prostate and a correlation with advanced stage, poorly differentiated and metastasis (13-14,22). Furthermore, P120ctn has been reported as an independent prognostic marker in gastroesophageal

adenocarcinoma (23). One possibility is that P120ctn absence from cells is tolerated in the context of the tumour microenvironment but not in vitro. Thus, signalling derived in vivo from tumour stroma or extracellular matrix may circumvent or substitute for a required P120ctn function (18). In vitro, such signals may be absent, such that cells explanted from P120ctn-deficient tumours fail to survive. Alternatively, the tumour microenvironment in vivo might actively suppress P120ctn expression, and the suppressive mechanism could be lost when the cells are cultured in vitro. Thus, explanting P120ctn-deficient tumour tissue to the culture dish might restore P120ctn expression. The resolution of these issues, and whether P120ctn expression is modulated by the microenvironment during tumour progression and metastasis, may provide clinically useful information relevant to understanding and managing metastasis. Recent identification and characterization of a unique P120ctn-deficient carcinoma cell line has provided the first molecular clues as to potential consequences of P120ctn loss in tumours (24-25). SW48 cells are colon carcinoma cells with mutated P120ctn genes and sharply reduced levels of P120ctn protein, providing a rare opportunity to examine the consequences of P120ctn loss and reconstitution in a tumour-derived cell line. Interestingly, the P120ctn deficiency appears to result in strongly reduced levels of E-cadherin, which in turn leads to loosely organized cells that fail to maintain epithelial morphology (26-27). Restoring p120 rescues the epithelial phenotype, apparently by stabilizing and restoring normal levels of E-cadherin. Thus, it is possible that morphologic and behavioural changes in some tumours are due to P120ctn loss and consequent destabilization of E-cadherin. All of these suggest that, P120ctn down-regulation constitute an important parameter for the diagnosis of patients with respect to tumour aggressiveness.

Conclusion

In conclusion, reduced expression of P120ctn mRNA and protein occurs frequently in pancreatic carcinoma, and is significantly correlated with invasion and metastatic potentia1 of tumour. Furthermore, expression of P120ctn may serve as a significant prognostic factor for survival in pancreatic carcinoma. These findings strongly suggest that P120ctn may be a novel molecular target for the diagnosis and treatment of pancreatic carcinoma.

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