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RESEARCH ARTICLE

Plasma and synovial fluid programmed cell death 5 (PDCD5) levels are inversely associated with TNF-α and disease activity in patients with rheumatoid arthritis

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Abstract

Context: Programmed cell death 5 (PDCD5), a novel apoptotic regulatory gene, has been reported to be associated with rheumatoid arthritis (RA) and osteoarthritis (OA), which can regulate the apoptosis of synoviocytes and chondrocytes cultured *in vitro*.
Objective: To study expression characteristic of PDCD5 in plasma and synovial fluid of RA patients, and analyze its correlation with tumor necrosis factor alpha (TNF-α) and disease activity in RA.
Methods: A total of 135 subjects were recruited into this study (44 RA patients, 46 OA patients and 45 healthy controls). PDCD5 and TNF-α concentrations in plasma and synovial fluid were analyzed by enzyme-linked immunosorbent assay.
Results: Plasma and synovial fluid PDCD5 concentrations were significantly elevated in RA patients. Pearson correlation analysis indicated that plasma and synovial fluid PDCD5 levels were inversely correlated with TNF-α. Moreover, plasma PDCD5 levels were also inversely correlated with C-reactive protein and erythrocyte sedimentation rate.
Conclusion: Plasma and synovial fluid PDCD5 could be useful for monitoring the activity and progression of RA, and its abnormal expression and dysfunction may be correlated to TNF-α in RA patients.

Keywords

Disease activity, PDCD5, rheumatoid arthritis, TNF-α

History

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Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory disease characterized by joint destruction and severe disability. Synovial hyperplasia is a hallmark of RA (Feldmann et al., 1996). This phenomenon appears to be associated with the enhanced levels of cytokines and relative lack of cell apoptosis. One of the primary cytokines is tumor necrosis factor α (TNF-α), which is produced primarily by activated macrophages and is capable of inducing fibroblast-like synoviocytes (FLS) proliferation, as well as the production of collagenase, stromelysin and other enzymes that promote invasion of cartilage and bone (Kim et al., 2000; Kobayashi et al., 1999). The ability of TNF-α to induce FLS proliferation and other biological effects is attributable to its potent gene-regulatory properties, which depend, at least in part, on its ability to activate nuclear factor κB (NF-κB) and Akt (Burow et al., 2000), and to induce mutation of p53 pathway (Schneider et al., 2010).

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Programmed cell death 5 (PDCD5) is cloned as a gene whose expression is increased during the apoptotic process of TF-1 cells induced by cytokine withdrawal using a cDNA-RDA method (cDNA representational differences analysis) (Liu et al., 1999). Previous studies have shown that the expression of PDCD5 was significantly down regulated in various human tumors, such as breast cancer (Hedenfalk et al., 2001), hepatocellular carcinoma (Xu et al., 2001) and chronic myelogenous leukemia (Ma et al., 2005). Aforementioned observations suggest that PDCD5 plays a significant role in both apoptotic and nonapoptotic programmed cell death and may participate in the pathophysiologic course of various diseases involving abnormal programmed cell death. Recent studies have shown that increased expression and nuclear translocation of PDCD5 could induce FLS apoptosis (Wang et al., 2007), which might realize its function as a co-activator of p53 to regulate transcription and cell cycle arrest (Xu et al., 2012).

Due to the positive and negative influence on cell proliferation and apoptosis, and their common p53 pathway, we find out whether there is any relationship between PDCD5 and TNF-α in RA, which is a problem of much research meaning. In addition, these key roles of PDCD5 and TNF-α in the balance between apoptosis and proliferation seem to be of potential relevance to the pathophysiology of RA, which

prompted us to speculate that PDCD5 may be responsible for the pathogenesis of RA, and may be correlated with the disease activity of RA patients.

To examine this hypothesis, we have analyzed the plasma and synovial fluid levels of PDCD5 in knee RA patients, OA patients and healthy controls. The objective of the present study was to evaluate both plasma and synovial fluid levels of PDCD5 in patients with knee RA, and determine the possible relationships between plasma and synovial fluid PDCD5 with TNF-α and disease activity of knee RA.

Materials and methods

Study subjects

Ethical approval for this study was granted by the ethics committee of Peking University People's Hospital. Written informed consent was obtained from the patients and healthy volunteers prior to their participation in this study. RA and OA patients were diagnosed according to the criteria of the American College of Rheumatology (Altman et al., 1986; Arnett et al., 1988). Patients who presented with obvious joint injury or infection were excluded from the study. Meanwhile, all cases were not treated with steroids, anti-TNF-alpha, chondroitin polysulfate or hyaluronic acid for at least one month before this study.

A total of 135 subjects were enrolled in the study (44 RA patients, 46 OA patients and 45 healthy controls). Body mass index (BMI) was calculated as weight in kilograms divided by height squared in meters (kg/m^2). The RA activity was determined using C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) routinely analyzed from peripheral blood obtained at the time of admission.

Laboratory analysis

Synovial fluid was obtained from patients undergoing arthroscopic operations and primary total knee arthroplasty, centrifuged to remove cells and joint debris and stored at −80 °C until the day of measurement. No synovial fluid was extracted from the controls due to ethical concerns. Venous blood samples collected from all participants were centrifuged and stored at −80 °C until utilized.

The concentrations of PDCD5 in plasma and synovial fluid were assayed by ELISA kits provided by Professor Yingyu Chen at Peking University Center for Human Disease Genomics with a lower limit of sensitivity of 1.56 ng/mL, and the intra- and inter-assay coefficients of variations were 5.51% and 3.86%, respectively. Standard curve showed a good linear relationship, the coefficients regression equation was $y = 0.003x + 0.129$ and the regression coefficient was 0.982. The concrete steps of this ELISA kits are as follows: plasma and synovial fluid samples were properly diluted (1:10) with phosphate buffered saline (PBS) containing 10 mg/mL bovine serum albumin (BSA), 100 μL of the diluted sample was added in duplicate into wells of ELISA plates which had been coated with mouse monoclonal antibody against human PDCD5. After 1 h incubation at 37 °C in a humid chamber, the plates were washed with Immunowash (BioRAD, Hercules, CA, Model 1575). Then properly diluted HRP-rabbit-anti-human PDCD5 IgG (1:2500) was added to each well and the plates were incubated at 37 °C in a humid chamber for 1 h. After a final washing with the washer, 3,3′,5,5′-tetramethyl benzidine (TMB) was added to develop the color. After incubation at room temperature for 10 min, the color reaction was stopped by the addition of H_2SO_4 (0.5 M) to each well and the absorbance was measured using a microplate reader (BioRAD, Model 680) at a wave length of 450 nm.

The concentrations of TNF-α in plasma and synovial fluid were measured using a commercially available highly sensitive ELISA kit from BioLegend (San Diego, CA). The range of detectable TNF-α was 7.8–500 pg/mL with an assay sensitivity of <1 pg/mL. All samples were diluted and assayed according to the manufacturer's instructions.

Table 1. Baseline clinical characteristics of knee RA patients, OA patients and controls. Data presented as mean ± SD.

Characteristics	Controls ($n = 45$)	RA ($n = 44$)	OA ($n = 46$)
Age (years)	51.3 ± 9.35	52.5 ± 13.4	66.0 ± 9.1*
Sex (F/M)	39/6	36/8	42/4
BMI (kg/m^2)	23.3 ± 3.5	23.0 ± 3.1	27.0 ± 3.4*
RF positivity (%)	NA	77.5	NA

RA, rheumatoid arthritis; OA, osteoarthritis; F, female; M, male; BMI, body mass index; RF, rheumatoid factor.
NA, not applicable.
*$p < 0.05$ compared with RA patients and controls.

Statistical analysis

All statistical analyses were performed with SPSS (Chicago, IL) software version 16.0 for Windows. Tests of normality and test of homogeneity of variances were employed to analyze the subject's age, BMI, PDCD5 and TNF-α concentration in the plasma and synovial fluid. When the populations from which the samples were normal or approximately normal distributed and the variances of the populations were equal, Student's *t*-test was performed to compare the means of two independent groups and one-way analysis of variance (ANOVA) was employed to compare the means of more than two independent groups. The correlations of plasma or synovial fluid PDCD5 concentrations with TNF-α, CRP and ESR were determined by Pearson's correlation analysis. Data were expressed as mean ± standard deviation (SD). *p* Values <0.05 were considered statistically significant for differences and correlations.

Results

Baseline clinical characteristics

The baseline clinical characteristics of the subjects are shown in Table 1. Difference in gender among RA patients, OA patients and healthy controls was not seen. Patients with OA had higher age and BMI than patients with RA and controls ($p < 0.05$). About 77.5% of patients with RA were RF positive. As demonstrated in Figure 1, RA patients had higher plasma PDCD5 concentrations compared to OA patients and controls (33.85 ± 23.55 versus 12.33 ± 9.96 and 17.41 ± 4.63 ng/mL; $p < 0.01$). Similarly, PDCD5 levels in synovial fluid were also higher than OA patients (45.94 ± 28.50 versus 19.30 ± 10.00 ng/mL; $p < 0.01$). Additionally, plasma PDCD5 levels showed a positive correlation with synovial fluid PDCD5 levels ($r = 0.686$, $p < 0.01$), and PDCD5 levels

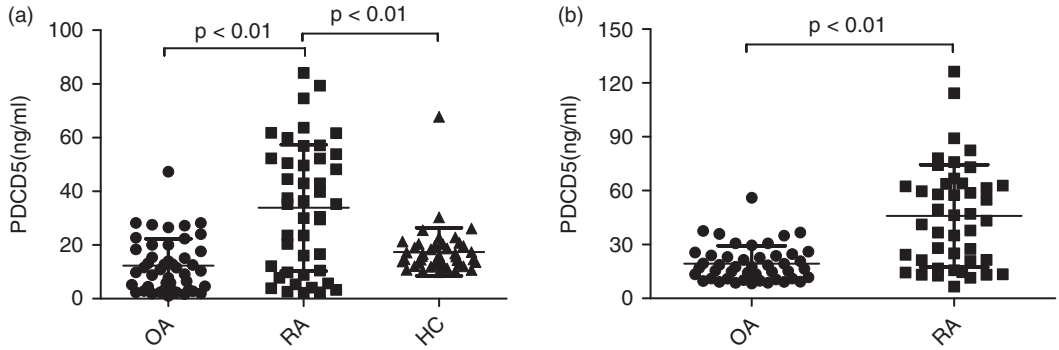

Figure 1. Over-expression of PDCD5 in plasma and synovial fluid of RA patients. (a) Plasma PDCD5 levels from patients with rheumatoid arthritis (RA; $n = 44$) versus osteoarthritis (OA; $n = 46$) and healthy controls (HC; $n = 45$), (b) synovial fluid PDCD5 levels from patients with RA ($n = 44$) versus OA ($n = 46$).

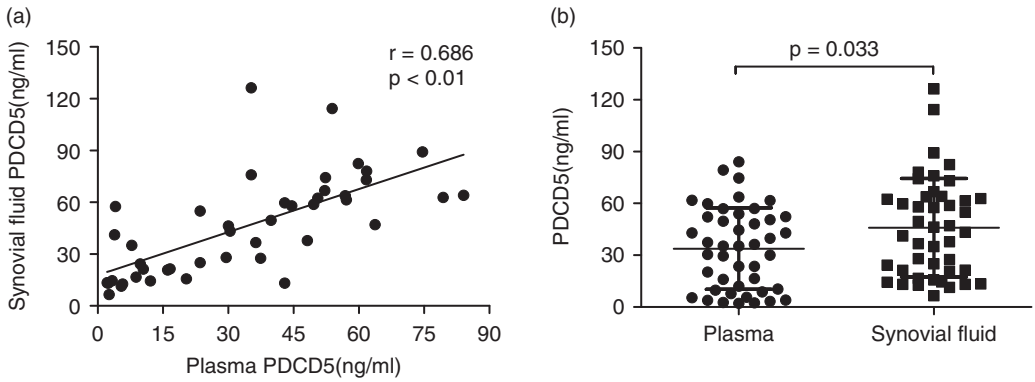

Figure 2. PDCD5 levels in plasma and synovial fluid of RA patients. (a) Scattergram showing the positive correlation between plasma and synovial fluid PDCD5 concentrations in RA patients ($r = 0.686$, $p < 0.01$), (b) PDCD5 levels were higher in synovial fluid compared to those in plasma of RA patients (33.85 ± 23.55 versus 45.94 ± 28.50 ng/mL; $p = 0.033$).

in synovial fluid were higher than in paired plasma samples (33.85 ± 23.55 versus 45.94 ± 28.50 ng/mL; $p = 0.033$) (Figure 2).

Association of plasma and synovial fluid PDCD5 levels with TNF-α

The TNF-α levels of plasma and synovial fluid in RA patients, OA patients and healthy controls are present in Table 2. The TNF-α levels of RA patients were significantly elevated compared with those of OA patients and healthy controls in both plasma and synovial fluid ($p < 0.001$). In RA patients, Pearson's correlation analysis revealed that plasma PDCD5 levels were significantly and negatively correlated with TNF-α ($r = -0.364$, $p = 0.014$) (Figure 3a). Additionally, synovial fluid levels of PDCD5 were also strongly negatively associated with TNF-α of RA patients ($r = -0.381$, $p = 0.010$) (Figure 3b).

Correlation of plasma PDCD5 levels with disease activity

There was no correlation of plasma and synovial fluid PDCD5 levels with age, BMI and gender distribution. To assess the relationship of PDCD5 with disease activity, we investigated the correlations among PDCD5, CRP and ESR using Pearson's correlation analysis. Our results showed that plasma PDCD5 level was inversely correlated with CRP and ESR ($r = -0.679$, $p < 0.01$ and $r = -0.720$, $p < 0.01$, respectively) (Figure 4a and b). But there was no correlation of synovial fluid PDCD5 levels with CRP and ESR.

Table 2. Plasma and synovial fluid TNF-α (pg/mL) in RA patients, OA patients and healthy controls. p Values for differences between RA patients and others. Data presented as mean ± SD.

	RA	OA	Controls	p Value
N	44	46	45	
Plasma	64.24 ± 25.90	20.56 ± 5.76	3.67 ± 4.22	<0.001
Synovial fluid	73.45 ± 26.56	22.51 ± 6.79	NA	<0.001

NA, not applicable.

Discussion

RA is a chronic immune-mediated disease marked by inflammation in the lining of the joint (i.e. the synovium) and destruction of cartilage and bone. Reduced apoptosis has been detected in synovial tissues from patients with RA. Insufficient apoptosis could potentially contribute to the synovial hyperplasia in RA, thereby enhancing focal production of inflammatory mediators and metalloproteinases that result in the destruction of cartilage and bone (Pope, 2002). Several genes have been implicated in reduced synoviocytes apoptosis, such as p53 (Seemayer et al., 2003), Bcl-2

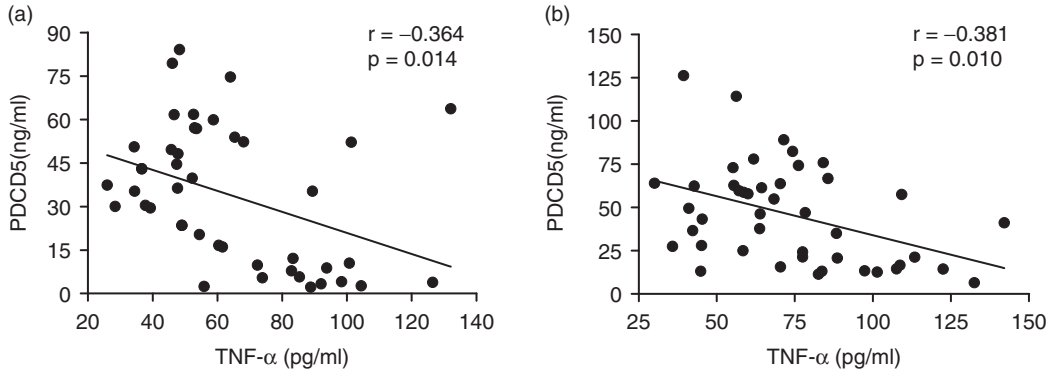

Figure 3. The relationships of PDCD5 and TNF-α in plasma and synovial fluid from RA patients. Correlations between PDCD5 and TNF-α in plasma of RA patients (a) and synovial fluid of RA patients (b). *r* Values of Pearson's product-moment correlation and *p* values of their null hypothesis are shown.

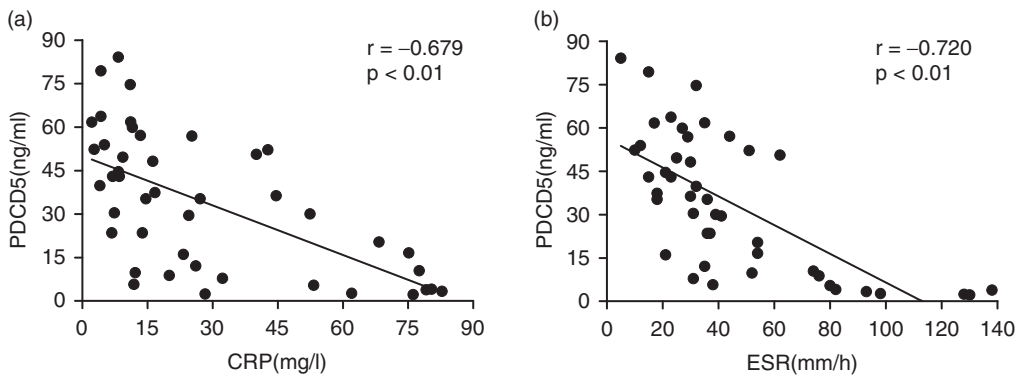

Figure 4. Correlation between PDCD5 and disease activity in RA patients. (a) Plasma PDCD5 levels negatively correlate with CRP ($r = -0.679$, $p < 0.01$), (b) plasma PDCD5 levels negatively correlate with ESR ($r = -0.720$, $p < 0.01$).

(Antonsson & Martinou, 2000) and FLIP (Irmler et al., 1997). For instance, Bcl-2 is more highly expressed in synovial tissue of RA and collagen-induced arthritis, particularly in FLS at sites of early erosion (Perlman et al., 2000, 2001). In the present study, we illustrated a significant elevation of PDCD5 concentrations in both plasma and synovial fluid of knee RA patients compared to OA patients and the control plasma concentrations. Our results indicated that there is increased local and systemic production of PDCD5 in knee RA. The results also revealed that PDCD5 levels in synovial fluid were higher than those observed in paired plasma samples, and there was a positive correlation between them. The higher PDCD5 levels in synovial fluid are presumably attributable to that PDCD5 was mainly synthesized in local tissues of RA knees, which could be originated from synovial cells and chondrocytes. Nevertheless, it cannot be a conclusion that abnormal PDCD5 expression only exists in local tissues of RA knees, which also might appear in extra-articular tissues. Previous studies have shown that PDCD5 was expressed in synovial tissue (Wang et al., 2007) and articular cartilage chondrocytes (Cheng et al., 2004). So further researches will be required to verify whether the concentrations of PDCD5 in synovial fluid and plasma are related to the local expression of PDCD5 in joint tissues.

Whether it can maintain a state of equilibrium between proliferation and apoptotic of synoviocytes is absolutely necessary for keeping the normal function of synovial tissue in the joint. An imbalance between proliferation, survival and death of synoviocytes may contribute to the occurrence and development of RA. According to their varied influences on synoviocytes proliferation and apoptosis, and the common signaling pathway of p53 (Schneider et al., 2010; Xu et al., 2012), studies on the relationship between TNF-α and PDCD5 in both plasma and synovial fluid might be useful to argue the origin of abnormal expression of PDCD5 in RA. The main finding of the present study was the demonstration of the inverse and independent relationship between PDCD5 and TNF-α in plasma and synovial fluid of RA patients. These results indicated that in the pathological joint environment, which has a complex milieu of cytokines, in addition to exerting its effects alone in pathophysiological process of RA, TNF-α appears to interact with a variety of proteins by affecting certain signaling pathway, which might influence PDCD5 expression in RA by interfering with certain signaling pathways. However, the present study has documented a significant elevation of both PDCD5 and TNF-α concentrations in plasma and synovial fluid of knee RA patients compared to OA patients and healthy controls. So the negative correlation between PDCD5 and TNF-α in RA patients may be resulted from the unbalanced increase of their expression. That is, the increase of TNF-α expression in RA patients could induce the elevation of PDCD5 expression through negative feedback of insufficient apoptosis in local and extra-articular tissues of RA knees, but the elevation of PDCD5 expression could not achieve the extent to antagonize the anti-apoptosis effect of TNF-α, or when PDCD5 expression have increased to a certain value, TNF-α would inhibit the expression of PDCD5 through some unclear mechanisms.

Further researches will be required to verify whether the change of TNF-α in synovial fluid or culture medium *in vitro* can influence the local expression of PDCD5 in joint tissues or vice versa.

The CRP and ESR are effective laboratory parameters to assess the activity of RA (Mallya et al., 1982). Another notable observation of the present study was to demonstrate that plasma PDCD5 levels were negatively correlated with the serum CRP and ESR concentrations of RA patients. These results revealed that the abnormal expression of PDCD5 might also play a crucial role in the progression of RA. This finding supports an additive role for plasma PDCD5 as a prediction factor of RA activity. However, due to the small sample size used by the study, the association between plasma PDCD5 levels and disease activity should be interpreted cautiously.

The potential limitations of the present study should be mentioned. First, the sample size is relatively small. Further study with a larger sample is required to determine the differences of plasma and synovial fluid PDCD5 levels among RA patients, OA patients and healthy controls. Second, only PDCD5 level has been measured in both plasma and synovial fluid. Further immunohistochemical studies of PDCD5 expression in local tissue could render more valuable information on the pathogenic role of PDCD5 in RA. Third, synovial fluid samples from healthy controls were not taken for ethical reasons. Finally, this study has been designed as a cross-sectional study; therefore, absolute cause and the effective relationship may not be possible. Future studies will focus on the interactions between PDCD5 and TNF-α to further elucidate the exact molecular mechanisms mediating the resistance to apoptosis in RA synoviocytes.

In conclusion, plasma and synovial fluid PDCD5 levels in patients with RA were significantly elevated compared with that of OA patients and healthy controls. Both plasma and synovial fluid PDCD5 levels were shown to be negatively correlated with the concentration of TNF-α, and plasma PDCD5 levels were also independently and negatively associated with the disease activity indexes in patients with RA. These findings suggest that PDCD5 and TNF-α proteins could interact with each other in the apoptosis of RA synoviocytes, and plasma PDCD5 might be a potential biomarker to reflect the disease activity and progression of knee RA.

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Declaration of interest

This research was facilitated by the National Natural Science Foundation of China (81071447). The authors report no conflicts of interest.

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