Research Communication

Diagnostic Investigations of DKK-1 and PDCD5 Expression Levels as Independent Prognostic Markers of Human Chondrosarcoma

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Abstract

In this study, we investigated the expression levels of Dickkopf-1 (DKK-1) and programmed cell death 5 (PDCD5) by using quantitative real-time PCR and immunohistochemistry in patients with chondrosarcoma. The DKK-1 mRNA levels were significantly higher in chondrosarcoma when compared with the corresponding nontumor tissues (mean ± SD: 4.23 ± 1.54; 1.54 ± 0.87; $P = 0.001$). PDCD5 mRNA levels were remarkably deceased in tumor tissues when compared with corresponding nontumor tissues (mean ± SD: 1.94 ± 0.73; 5.42 ± 1.73; $P = 0.001$). The high and moderate DKK-1 expressions were observed for 60% of chondrosarcoma samples in comparison with 27.5% of corresponding nontumor tissues ($P = 0.001$). Moreover, low expression of PDCD5 was found in 67.5% of the tumor tissues when compared with the nontumor tissues (32.5%; $P = 0.002$). The results of this study showed that high DKK-1 expression levels were strongly related to MSTS stage ($P = 0.011$) and the advancement of histological grade ($P < 0.001$). Furthermore, the PDCD5 expression levels were correlated with histological grade ($P < 0.001$), MSTS stage ($P = 0.016$), and distant metastasis ($P = 0.001$). Kaplan-Meier survival and log-rank survival showed that patients with high DKK-1 levels and low PDCD5 levels were correlated with shorter overall survival (log-rank test $P < 0.001$). PDCD5 levels, histological grade, and tumor stage were independent predictors of overall survival. In conclusion, DKK-1 and PDCD5 can be independent predictors of overall survival in patients suffering from chondrosarcoma.

Keywords: *DKK-1 and PDCD5; chondrosarcoma; immunohistochemistry; patients; PCR*

Volume 68, Number 7, July 2016, Pages 597–601
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Received 28 April 2016; Accepted 14 May 2016
DOI 10.1002/iub.1519
Published online 3 June 2016 in Wiley Online Library (wileyonlinelibrary.com)

Introduction

Chondrosarcoma is a malignant cartilage-forming tumor (1–3), and patients with chondrosarcoma are resistant to both chemotherapy and radiation treatments, although surgical resection is still the important treatment. The prognosis of disease is strongly linked to tumor grade and metastatic status with high morbidity, and 10-year survival rate has been showed to be between 29 and 83% (2,4). Therefore, there is an urgent need to identify biomarkers, the molecular mechanisms, and therapeutic targets for

chondrosarcoma. Dickkopf-1 (DKK-1) is an important regulatory factor of Wnt signaling pathway, which can disrupt Wnt-induced Fz-LRP6 complex formation DDK-1 and contribute to inhibition of Wnt signaling (5,6). The expression level of DKK-1 is different in many types of cancers, indicating that it may have tumor-specific role. DKK-1 overexpression was found in many malignant tumors, including breast cancer hepatoblastomas, lung cancer, esophageal carcinomas, Wilm's tumors, and multiple myeloma (7–11), suggesting a potential oncogenic function of DKK-1. However, contradicting results have been found in other investigations. Its expression was reduced in melanoma cells, renal clear cell carcinoma, gastric cancer, and colon cancer (12,13). High expression of DKK-1 was detected in early prostate cancer, whereas it was decreased during progression (14). It has been indicated that DKK-1 can be as a prognostic biomarker and may be associated with bone metastases of breast and prostatic carcinomas (11,15,16). Programmed cell death 5 (PDCD5) can accelerate DNA damage-induced apoptosis with the histone acetyl transferase Tip60 and phosphorylated by the multifunctional kinase CK2 (17). PDCD5 fragments have been found to be associated with suppression of the tumorigenesis via inhabitation of the Ras/Raf/MEK/ERK signaling pathway. Decreased expression of PDCD5 was found in many kinds of malignancies such as ovarian carcinomas, lung cancer, bladder carcinoma prostate cancer, chondrosarcoma, bladder carcinoma, colorectal cancer, renal clear cell carcinoma, gliomas, myeloma, and acute myeloid leukemia (18–21). Low PDCD5 expression level may be involved in tumor progression and prognosis. We investigated the expression levels of DKK-1 and PDCD5 and their clinical importance in patients with chondrosarcoma.

Materials and Methods

Clinical Specimens

Primary chondrosarcoma of bone specimens and corresponding nontumor tissues were collected between 2008 and 2012 from 40 patients who were undergoing surgery in Tehran hospitals, Iran. The patients were not previously treated with chemotherapy or radiotherapy before operation. Moreover, the tissues were reviewed by pathologists to confirm the histologic diagnosis.

Quantitative Real-Time PCR

Total RNA was isolated from tissues using RNA extraction kit (CinnaPure-RNA Cell Culture, Bacteria, Tissues and Plasma, Iran). A poly(dT) oligonucleotide was used as the primer to generate cDNA by reverse transcription (Invitrogen). QRT-PCR analyses performed using TaqMan universal PCR master mix with Applied Biosystems 7500 real-time PCR system. GAPDH was applied as control, and fold changes were analyzed using the $2^{-\Delta\Delta CT}$ method.

Immunohistochemistry

Slides were cut to a thickness of 4-μm sections from paraffin-embedded specimens. The sections were incubated with 3% H_2O_2 in 100% methanol to block the endogenous peroxidase activity and followed by 5% blocking serum in PBS for 30 min. The sections were incubated with primary anti-DKK-1 antibody (1:200; Abcam) and anti-PDCD5 antibody (1:200 dilution). The slides were incubated with 1:30 dilution biotin-labeled secondary antibodies (Sigma) and followed by further incubation with streptavidin-horseradish peroxidase for 20 min. 3′-Diaminobenzidine substrate was applied as chromogene. The percentage of positive cells was also scored as follows: negative (−): 0–5%; weak: 6–25%; moderate: 25–50%; and strong: 50–100%. Cell staining was categorized into high-expression (3–9) and low-expression (0–2) groups.

Statistical Analysis

All variables were evaluated using the SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was evaluated using χ^2 test. The log-rank test and Kaplan-Meier analysis were used for survival analysis. Prognostic values were analyzed by univariate and multivariate Cox regression analyses. $P < 0.05$ was considered to be statistically significant.

Results

DKK-1 and PDCD5 Levels in Chondrosarcoma Tissues

The clinicopathological factors are given in Table 1. The DKK-1 mRNA levels were upregulated in chondrosarcoma when compared with the corresponding nontumor tissues (mean ± SD: 4.23 ± 1.54; 1.54 ± 0.87; $P = 0.001$; Fig. 1). PDCD5 mRNA levels were remarkably decreased in chondrosarcoma tissues when compared with corresponding nontumor tissues (mean ± SD: 1.94 ± 0.73; 5.42 ± 1.73; $P = 0.001$; Fig. 2).

The positive staining of DKK-1 was found in the cytoplasm, and high and moderate DKK-1 expressions were observed in 60% (24/40) of chondrosarcomas samples in comparison with 27.5% of (11/40) corresponding nontumor tissues ($P = 0.005$). Moreover, the positive staining of PDCD5 was seen in the cytoplasm, and low expression of PDCD5 was found in 67.5% of the cytoplasm of the tumor tissues (27 cases) when compared with the nontumor tissues (32.5%, 13 cases; $P = 0.002$).

Association of DKK-1 Level and PDCD5 with Clinicopathogical Parameters

The results of this study showed that high DKK-1 expression was related to MSTS stage ($P = 0.011$) and the advancement of histological grade ($P < 0.001$). Furthermore, the PDCD5 expression levels were associated significantly with histological grade ($P < 0.001$), MSTS stage ($P = 0.016$), and distant metastasis ($P = 0.001$; Table 1).

Association of Genes Expression with Survival

Kaplan-Meier survival and log-rank survival analysis showed that patients with high DKK-1 levels and low PDCD5 levels were related to poorer overall survival ($P < 0.001$).

Multivariate Cox proportional hazards model indicated that DKK-1 level, PDCD5 levels, histological grade, and tumor stage were independent predictors of overall survival (Table 2).

TABLE 1 ***Association of DKK-1 level and PDCD5 levels with characteristics of chondrosarcoma***

Clinicopathological features	*Number of cases*	*Expression of DKK-1*		*Expression of PDCD5*		P-*value of DKK-1*	P-*value of PDCD5*
		Low	*High*	*Low*	*High*		
Gender		16	24	13	27		
Male	22	7	15	8	14	0.427	0.782
Female	18	9	9	5	13		
Age (years)							
≤40	14	5	9	5	9	0.614	0.645
>40	26	11	15	8	18		
Primary site							
Pelvis	12	4	8	3	9	0.643	0.623
Scapula	14	6	8	4	10		
Vertebra							
Femur	7	2	5	2	5		
Rib	3	2	1	2	1		
Tibia	2	1	1	1	1		
Knee	2	1	1	1	1		
Histological grade							
1	18	8	10	7	11	< 0.001	< 0.001
2	10	5	5	4	6		
3	12	3	9	2	10		
Distant metastasis							
No	26	10	16	12	14	0.427	0.001
Yes	14	6	8	1	13		
MSTS stage							
Ia	12	9	3	5	7	0.011	0.016
Ib	10	4	6	5	5		
IIa	7	2	5	1	6		
IIb	11	1	10	2	9		

Discussion

DKK-1 overexpression was found in many malignant tumors, including breast cancer hepatoblastomas, lung cancer, esophageal carcinomas, Wilm's tumors, and multiple myeloma (7–11), suggesting a potential oncogenic function of DKK-1. However, contradicting results have been found in other investigations. The reduction of DKK-1 was found in melanoma cells, renal clear cell carcinoma, and colon cancer (12,13). High expression of DKK-1 was detected in early prostate cancer, whereas it was decreased during progression (14). It has been indicated that DKK-1 can be used as a prognostic biomarker and may be associated with bone metastases of breast and prostatic carcinomas ((11), (15), (16)). In the current study, the DKK-1 mRNA and protein expression were

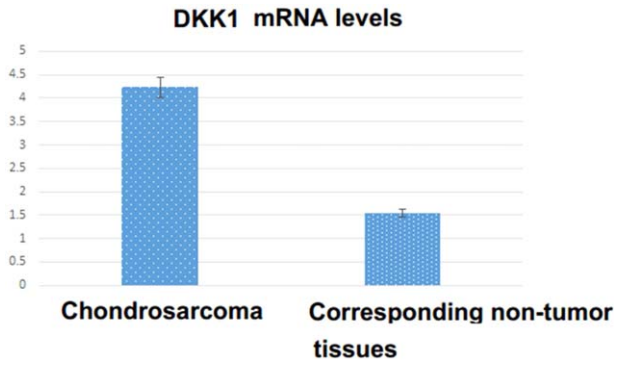

FIG 1 *The DKK-1 mRNA levels in chondrosarcoma when compared with the corresponding nontumor tissues.*

markedly elevated in chondrosarcoma when compared with the corresponding nontumor tissues. Current evidence showed that the functions of DKK-1 may be correlated with cancer type and the tissue microenvironment. These findings show that high DKK-1 expression was significantly related to MSTS stage and the advancement of histological grade. Kaplan-Meier survival and log-rank survival analysis showed that patients with high DKK-1 levels had shorter overall survival (log-rank test $P < 0.001$). Multivariate Cox proportional hazards model indicated that DKK-1 levels, histological grade, and tumor stage were independent predictors of overall survival. Xiang et al. (22) reported that DKK-1 expression increased in lung cancer cell lines, and its expression was transactivated in these cancer cell lines. Furthermore, it was suggested that DKK-1 play an important regulatory role in the progression of NSCLC.

In accordance with our survey, Chen et al. (23) reported that both DKK-1 and β-catenin levels were in high level in chondrosarcoma when compared with nontumor tissues. Furthermore, DKK-1 and β-catenin levels were remarkably linked to overall survival. The association of increased DKK-1 levels with β-catenin accumulation has been indicated in chondrosarcoma, and its expression was correlated with poor prognosis in patients with chondrosarcoma (23).

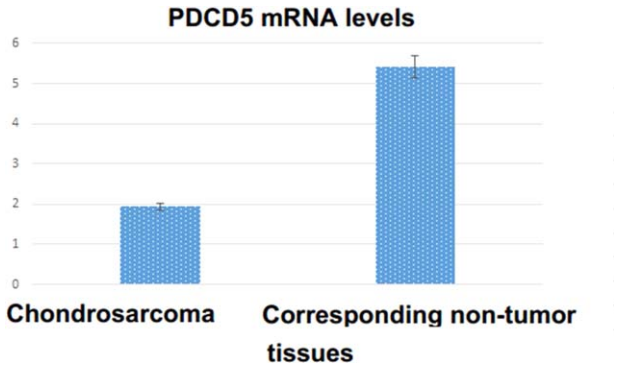

FIG 2 *The PDCD5 mRNA levels in chondrosarcoma when compared with the corresponding nontumor tissues.*

TABLE 2 ***Multivariate analysis of overall survival by Cox regression model***

Clinicopathological characteristics	*HR*	*95% CI*	P-*value*
Gender	0.743	0.343–1.3231	0.723
Age	0.84	0.382–1.522	0.682
Tumor stage	3.242	1.923–10.23	0.001
Location	0.623	0.362–2.321	0.742
Distant metastasis	1.871	1.331–3.126	0.257
Tumor grade	2.743	1.621–9.441	0.021
PDCD5 expression	3.461	1.851–11.33	0.003
DKK-1 expression	2.701	1.581–9.138	0.032

Abnormal PDCD5 expression is linked to many diseases. Decreased expression of PDCD5 was found in many kinds of malignancies such as ovarian carcinomas, lung cancer, bladder carcinoma prostate cancer, chondrosarcoma, bladder carcinoma, colorectal cancer, renal clear cell carcinoma, high-grade astrocytic gliomas, myeloma, and acute myeloid leukemia (18–21). Low expression of PDCD5 has been shown to be correlated with tumor progression and prognosis. Our result suggested that PDCD5 mRNA and protein levels were remarkably deceased in chondrosarcoma tissues in comparison with the corresponding nontumor tissues. Our results showed that PDCD5 expression levels were linked to histological grade, tumor stage, and distant metastasis. Kaplan-Meier survival and log-rank survival analysis showed that patients with low PDCD5 levels were correlated with shorter overall survival (log-rank test $P < 0.001$). Multivariate Cox proportional hazards model indicated that DKK-1 level, PDCD5 levels, histological grade, and tumor stage were independent predictors of overall survival.

PDCD5 expression was strongly decreased in tumor cells when compared with normal cells and associates with malignancy, tumor stage, and prognosis ((18), (24–26)). In a study, Yang et al. (27) demonstrated that low PDCD5 expression was highly linked to poor prognosis for clear cell kidney cancer, gastric cancer, and chondrosarcoma. These investigations suggested that PDCD5 protects against tumorigenesis and tumor progression. Furthermore, Ye and coworkers (28) expressed that low PDCD5 expression was also inversely correlated to FIGO scores and that higher PDCD5 expression is linked to longer survival times for patients with ovarian cancer. Furthermore, it has been suggested that low PDCD5 expression can be strongly linked to anatomical location and histological grade. Poor survival is reported to be correlated with low expression of PDCD5, which is an independent prognostic factor for overall survival (19). In conclusion, DKK-1 and PDCD5 can be independent predictors of overall survival in patients with chondrosarcoma.

Conflict of Interest

The authors confirm that this article content has no conflicts of interest.

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