

CD151 Protein Expression Predicts the Clinical Outcome of Low-Grade Primary Prostate Cancer Better than Histologic Grading: A New Prognostic Indicator?

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Abstract

Objective: CD151 is the first member of the tetraspanin family to be associated as a promoter of human tumor metastasis. However, its biological function and expression phenotype among different tumors has not been well investigated.

Method: Tissue specimens from 76 primary prostate cancers and 30 benign prostate hyperplasia (BPH) controls were obtained from the Department of Anatomical Pathology at the Austin and Repatriation Medical Centre (now Austin Health) from 1984 to 1993. We used quantitative immunohistochemical analysis to measure CD151 protein expression. Analyses of differences among BPH and prostate cancer groups were done with one-way ANOVA and Newman-Keuls test. The Kaplan-Meier method and the log-rank test were used to estimate the overall survival.

Results: CD151 expression was found to be significantly higher in prostate cancer specimens compared with BPH specimens ($P < 0.001$). Poorly differentiated

cancers expressed the strongest staining, whereas well-differentiated cancers expressed the weakest staining for CD151 ($P < 0.001$). The overall survival rate for cases in which CD151 expression was reduced was significantly higher than for cases in which CD151 expression was increased ($P = 0.039$) especially in well and moderately differentiated cancers ($P = 0.014$). This effect was independent of the patients' age or preoperative prostate-specific antigen values and superior in the predictive ability of the Gleason score.

Conclusions: CD151 has an increasing expression pattern in prostate cancer progression, and higher levels of CD151 are associated with poorer prognosis. CD151 had better predicting value for the clinical outcome of prostate cancer patients than does the traditional histologic grading method (Gleason grading). (Cancer Epidemiol Biomarkers Prev 2004;13(11):1717-21)

Introduction

Prostate cancer is the most commonly diagnosed cancer in developed countries. Despite its high morbidity, only a relatively small proportion of patients suffering from prostate cancer will die from the disease (1). Estimation of prognosis and life expectancy is therefore important in choosing the initial treatment strategy and judging the likely outcome of the individual patient.

Some tetraspanin family members including CD9 (2), CD63 (3), and CD82 (4) have been described as suppressors of tumor metastasis. Interestingly, CD151 and CO-029 are the only two members of the tetraspanin family that have been identified as promoters of metastasis (5-7).

CD151, also known as PETA-3 or SFA-1, is located on chromosome 11p15.5 (8, 9). CD151 is widely expressed by keratinocytes, endothelial and epithelial cells, smooth

muscle, cardiac muscle, and lymphocytes (10). CD151 gene and protein expression in tumor tissues from lung and colon cancer patients have been investigated recently (11, 12); higher levels of CD151 expression were found to foreshadow a poorer prognosis. For further investigation into the role of CD151 in tumor metastasis, researchers transfected CD151 cDNA into different tumor cell lines and found that these cells overexpressing CD151 were more aggressive than the control cells with enhanced motility and invasion (5, 13).

Because CD151 is involved in cancer cell movement and metastasis, it may be a potentially valuable marker as a prognostic factor in predicting the clinical behavior of cancer. We therefore investigated the levels of CD151 protein expression in patients with primary prostate cancer without associated metastasis and correlated this protein expression with the clinical outcome in these patients in comparison with the traditional histologic grading method (Gleason grading).

Materials and Methods

Subjects and Biopsies. Formaldehyde-fixed, paraffin-embedded human prostate tissue specimens were

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obtained from 76 patients with primary prostate cancer from 1984 to 1993 at the Austin and Repatriation Medical Centre (now Austin Health). This study was conducted with the approval of Austin and Repatriation Medical Centre Ethics Committee. Patient surgical specimens included 19 well-differentiated (Gleason grade 2-4), 34 moderately differentiated (Gleason grade 5-7), and 23 poorly differentiated (Gleason grade 8-10) prostate cancers (14) without preoperative evidence of metastatic disease and without previously exposing to systemic or hormonal therapy. As controls, 30 cases of benign prostate hyperplasia (BPH) surgical specimens were used. All the specimens were examined microscopically, and all cancer cases were classified histologically according to the Gleason system (15). Postoperative details were available from Urology outpatient attendance for these patients. Thirty of the 76 primary prostate patients were used in the survival statistics. The other 46 patients died from diseases other than prostate cancer or were lost after follow-up. The median follow-up for all cancer patients was 40 months.

Immunohistochemistry. CD151 protein expression in tissue sections was measured by immunohistochemistry using DAKO LSAB+ kit (DAKO Corp., Carpinteria, CA). The epitope retrieval was done by heating in 10 mmol/L citric acid (pH 6.0, Sigma Chemical Co., St. Louis, MO) buffer bath for 10 minutes in the microwave. Sections were incubated for 2 hours with monoclonal mouse anti-human CD151 antibody (11B1, purified immunoglobulin IgG2a, 4 µg/mL working concentration; ref. 10). The antibody was detected by incubating with anti-mouse biotinylated link antibody and peroxidase-labeled streptavidin (DAKO) for 30 minutes each. The antigen-antibody reaction complex was visualized by 3,3'-diaminobenzidine (DAKO) solution. All sections were then counterstained with hematoxylin for 10 seconds. Isotype antibody 1D4.5 (IgG2a; ref. 10) was used as negative control. All those steps were carried out under room temperature.

Immunohistochemical Quantification. Immunohistochemistry results were judged based on the intensity of staining (16). The M2 program of the microcomputer imaging device (Imaging Research, Inc., St. Catharines, Ontario, Canada) was used to quantify the staining. The CD151 expression was measured without prior knowledge of the grade or patient survival. Brown staining of 3,3'-diaminobenzidine was calculated with the following variable settings: scan area, hue 0.00-40.78 and 303.75-359.00; intensity 0.000-0.702; and saturation 0.00-1.00. The density and area were measured for each digitized image. Each sample window was set to 20 × 20 µm, which is half the size of an average epithelial cell. Only cytoplasmic regions of the epithelial cell were measured. Twenty windows within 10 adjacent fields in each specimen at a magnification of 200× were measured (17). Density and area were multiplied to represent the intensity of CD151 staining for each specimen.

Statistical Analysis. Analyses of differences among BPH specimens, prostate cancer specimens, and three histologic differentiated specimens were done with one-way ANOVA and Newman-Keuls test. Multiple regression analysis was used to measure the correlation between variables and CD151 protein expression. The

Kaplan-Meier method was used to estimate the probability of overall survival. The prognostic significance was evaluated by the log-rank test. All *P*s were based on two-tailed statistical analysis, and *P* < 0.05 was considered to indicate statistical significance.

Results

Images of CD151 protein staining in representative samples of well, moderately, and poorly differentiated cancer specimens, BPH surrounding cancer (BPH areas in tumor specimens), and BPH control (normal BPH specimens) are shown in Fig. 1.

CD151 protein was expressed in prostatic epithelial cells of tumor specimens and BPH specimens. Prostate cancer specimens expressed higher content of CD151 compared with BPH control and BPH surrounding cancer specimens (both *P* < 0.001), with no significant difference being found between BPH control and BPH surrounding cancer specimens (*P* > 0.05; Fig. 2). The CD151 expression varied substantially among prostate cancer specimens. Well-differentiated cancer specimens expressed the weakest staining of CD151 protein. Neither BPH control nor BPH surrounding cancer showed significant differences to well-differentiated cancer specimens (*P* = 0.19 and 0.40, respectively). According to patients' histologic grading, CD151 expression increased with increased grading of cancer. The difference among the three histologic groups was significant (*P* < 0.0001; Fig. 3).

Multiple regression analysis of variables associated with CD151 protein expression in patients with prostate cancer is shown in Table 1. Age and prostate-specific antigen values were not found to correlate with CD151 expression (*P* = 0.70 and 0.34, respectively), whereas Gleason grading and differentiation were positively correlated with CD151 expression among prostate cancer patients (both *P* < 0.0001).

Gleason grading is a common method to classify prostate cancer patients and to predict clinical outcome (15). We divided prostate cancer patients into Gleason grade ≥7 group and <7 group (16). Although there was a trend toward better survival with lower Gleason scores, as might be expected, we found no significant difference

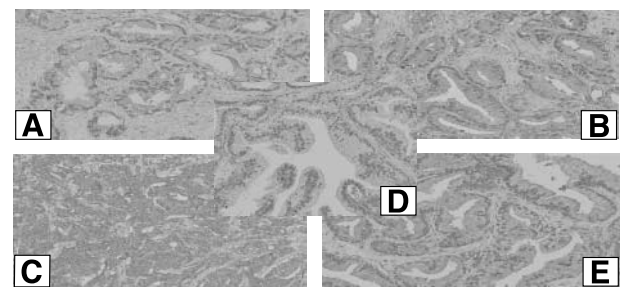


Figure 1. Expression of CD151 protein in BPH control, BPH surrounding cancer, and prostate cancer specimens. **A.** Well-differentiated cancer specimen. **B.** Moderately differentiated cancer specimen. **C.** Poorly differentiated cancer specimen. **D.** BPH control specimen. **E.** BPH surrounding cancer specimen.

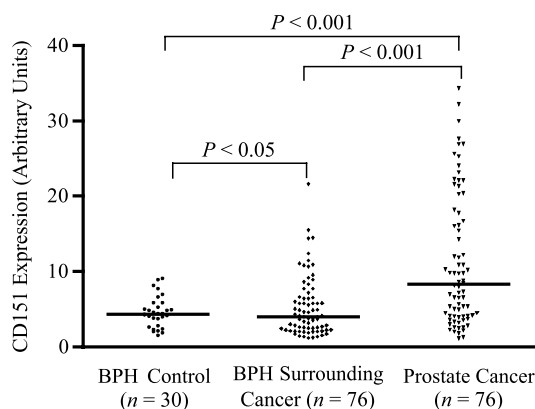


Figure 2. The CD151 expression in BPH and prostate cancer specimens. The CD151 expression among prostate cancer specimens varied from 1.15 to 34.35, with a median of 8.31; the CD151 expression in BPH control specimens ranged from 1.45 to 9.03, with a median of 4.37; and the CD151 expression in BPH surrounding cancer specimens ranged from 1.17 to 21.62, with a median of 4.03.

of survival time between these two groups not only in all patients but also in well and moderately differentiated cancer patients ($P = 0.17$ and 0.46 , respectively; Fig. 4A and B).

Thirty primary prostate cancer patients were divided into two groups using the third quartile (17.52) of all scores of prostate cancer CD151 expression as the cutoff point. Twenty-three (77%) patients with CD151 expression lower than 17.52 were nominated as CD151 "reduced expression," whereas 7 (23%) patients with CD151 expression higher than 17.52 were nominated as CD151 "increased expression." The CD151 reduced

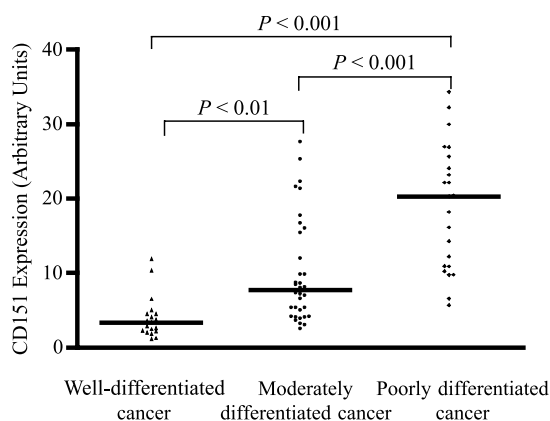


Figure 3. The CD151 expression in three histologic differentiated prostate cancer specimens. Well-differentiated cancer specimens expressed the weakest staining of CD151 protein (median, 3.35; range, 1.15-10.33). Elevated expression of CD151 was observed in the moderately differentiated cancer specimens (median, 7.68; range, 2.55-27.65). The strongest CD151 expression was found in the poorly differentiated cancer specimens (median, 20.28; range, 5.69-34.35).

expression specimens showed more heterogeneous staining compared with the CD151 increased expression specimens, as seen in non-small cell lung cancer (11), although the basis for these differences is not known. The median survival time of the CD151 increased expression patients was 26 months compared with 99 months of the CD151 reduced expression patients. As shown in Fig. 4C, patients with reduced CD151 expression had a better prognosis ($P = 0.039$).

We further divided all cancer patients according to levels of differentiation (Fig. 4D and E). Our analysis found that patients with reduced expression of CD151 had a better prognosis compared with those with increased expression of CD151 in well and moderately differentiated cancer patients ($n = 21$; $P = 0.014$). The median survival time of the CD151 increased expression patients was 12 months compared with 99 months of the CD151 reduced expression patients. There was no significant difference between these two groups in poorly differentiated cancer patients ($P = 0.83$).

Discussion

CD151 cDNA was originally isolated from the megakaryoblastic cell line MO7e clone and termed PETA-3 in 1995 (9). Although the exact biochemical function of CD151 is still unknown, evidence shows that it is a crucial member in signal transduction (18, 19), cell adhesion (20), and motility (5, 13, 19).

CD151 was said to be involved in an early step in the formation of secondary metastatic lesions, and its role in tumor dissemination is probably due to its ability in mediating cell migration (5). The increase of cell migration caused by CD151 cDNA transfection was inhibitable by anti-CD151 antibody (5, 13). Longo et al. (21) studied the distribution of several tetraspanins using tumor cell and endothelial cell mosaic monolayers grown on two-dimensional collagen. CD151 was found to be concentrated at the tumor cell-endothelial cell contact regions, suggesting the possible role of CD151 in molecular interactions required for transendothelial invasion by tumor cells.

Like other tetraspanin family members, CD151 can complex with integrins (19, 22-25). The integrins, which serve as linkages between extracellular matrix and structural elements inside the cell, are also essential for cell adhesion, migration, and apoptosis (26). CD151 stands out among all tetraspanins because of its crucial role in the tetraspanin-integrin network. CD151 interacts directly with integrins $\alpha_3\beta_1$ and $\alpha_6\beta_1$, and it is likely that other tetraspanins interact indirectly with integrins

Table 1. Multiple regression analysis of variables associated with CD151 protein expression in patients with prostate cancer

Variables	Standardized coefficient	P
Age	-0.046	0.70
Prostate-specific antigen value	0.245	0.34
Gleason grading	0.624	<0.0001
Differentiation	0.639	<0.0001

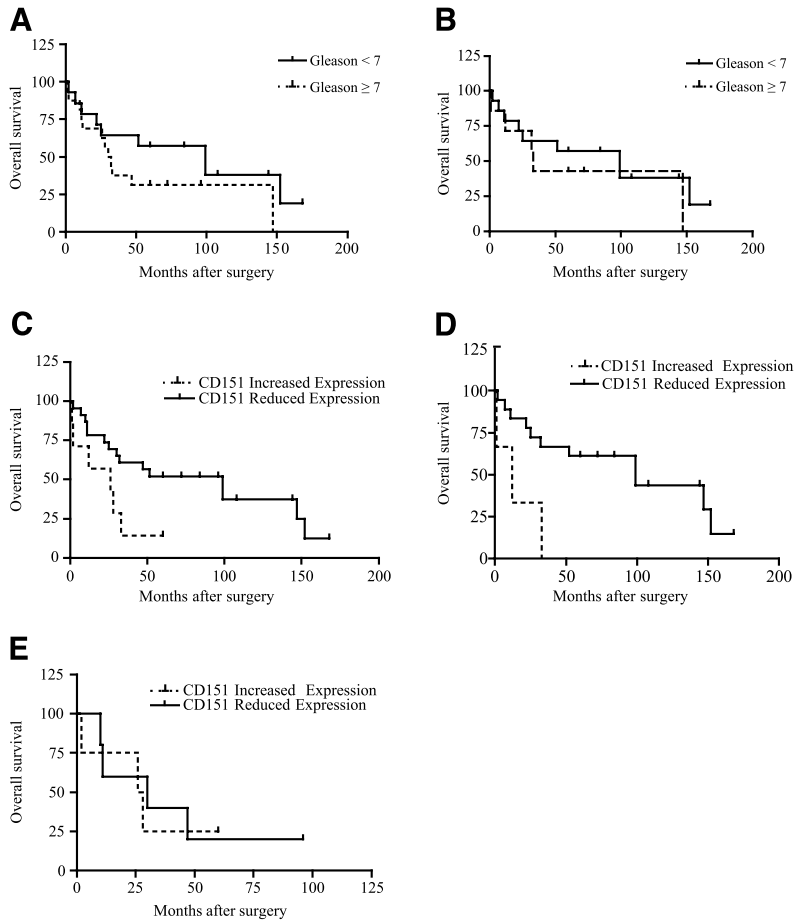


Figure 4. The survival rate comparisons in patients with primary prostate cancer. **A.** For all prostate cancer patients ($n = 30$), there was no statistical significance between high Gleason grading group (≥ 7 , $n = 16$) and low Gleason grading group (< 7 , $n = 14$; $P = 0.17$). **B.** For well/moderately differentiated cancers ($n = 21$), there is no significant difference between these two groups either ($n = 7$, $n = 14$; $P = 0.46$). **C.** The overall survival of total 30 prostate cancer patients with increased CD151 expression or reduced CD151 expression. The CD151 reduced expression group (*CD151-R*; $n = 23$) had a better prognosis than that of the CD151 increased expression group (*CD151-I*; $n = 7$; $P = 0.039$). **D.** Overall survival of well/moderately differentiated cancers ($n = 21$). The CD151 reduced expression group ($n = 18$) had a better prognosis compared with that of the CD151 increased expression group ($n = 3$; $P = 0.014$). **E.** Overall survival of poorly differentiated cancers. No statistical significance was found between CD151 reduced expression group and CD151 increased expression group ($n = 9$; $P = 0.83$).

through interactions with CD151 (23). There is a highly stoichiometric, stable, specific, and direct association between integrin $\alpha_3\beta_1$ and CD151, which is functionally relevant, and such association can be observed even in relatively stringent detergents (19). CD151 may recruit signaling enzymes into tetraspanin-integrin complexes (27); however, the exact function of CD151 in the complex is still unknown.

Despite these findings, the pattern of CD151 distribution among different tumor types has not been well investigated in the past. Our data show that CD151 expression was quite low in epithelial cells in BPH, whereas expression was much higher in primary prostate cancer specimens. In the present work, we found that CD151 protein expression was positively correlated with cancer differentiation according to Gleason grading (15), which is the most well-established pathologic criterion for judging the clinical stage and malignant progression in prostate cancer. Our study revealed that the poorer the differentiation of the cancer, the stronger the expression of CD151 seen, there being a significant difference among well, moderately, and poorly differentiated prostate cancer patients.

The present study found that CD151 expression was negatively correlated with the survival time of primary prostate cancer patients. The overall survival in patients in whom CD151 expression was reduced was signifi-

cantly higher than that of patients in whom CD151 expression was increased, consistent with the findings in the lung and colon cancer patients (11, 12). Importantly, CD151 showed a better predictive value than Gleason grading, although there was a trend that patients with low Gleason grade had a better prognosis than that of patients with high Gleason grade. There was a strong correlation between survival according to the CD151 tumor content, especially in patients with well or moderately differentiated cancers. Detection of CD151 expression might therefore be more valuable in predicting prognosis and choosing suitable therapies for individual prostate cancer patients than histologic grading alone.

Improvements in diagnosis and monitoring of the clinical behavior of prostate cancer have occurred since the widespread use of prostate-specific antigen screening and the combination of different diagnostic methods. However, clinicopathologic variables, including biopsy features and serum prostate-specific antigen, are often insufficient to predict tumor growth potential and the prognosis for individual patients (28, 29). More accurate predictors of clinical outcome of prostate cancer patients are therefore needed. Our present results show that higher levels of CD151 protein expression are associated with poorer prognosis in primary prostate cancer patients, an association not seen as strongly as with the

histologic grading of the primary tumor. Therefore, CD151 may be a more valuable marker in predicting the outcome of primary prostate cancer, especially in well and moderately differentiated cancer patients (Gleason grade 2-7), which have typically less predictable clinical outcomes when assessed by histologic grading.

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