Original paper

Expression of stem cell marker CD44 in prostate cancer biopsies predicts cancer grade in radical prostatectomy specimens

Konstanty Korski[1], Anna Malicka-Durczak[2], Jan Bręborowicz[1]

[1]Department of Pathology, The Greater Poland Cancer Centre, Poznan, Poland
[2]Poznan University of Medical Sciences, Poznan, Poland

Cancer stem cells play an important role in development and progression of many cancer types including prostate adenocarcinoma. We used a stem cell marker CD44 to evaluate the prevalence of prostate cancer stem cells in prostate biopsies and in matched radical prostatectomy specimens.
We tested both types of specimen for the existence of a correlation between the immunohistochemical expression of CD44 and Gleason grade, pathological stage (pT) according to TNM, patient age and preoperative plasma PSA levels in 52 patients.
We found a positive correlation between the expression of CD44 in cancer cells from prostate biopsies and in matched radical prostatectomy specimens. We also observed that higher level of CD44 expression in cancer cells correlated with lower Gleason score, both in prostate biopsies and in radical prostatectomies.
To the best of our knowledge we showed for the first time, that the level of CD44 expression in prostate biopsies correlates with that observed in matched radical prostatectomy specimens. Since the level of CD44 expression was shown to predict a response to anti cancer therapy in several types of human tumors, CD44 assessment might support a clinical decision making process in prostate cancer patients.

Key words: prostate cancer, cancer stem cell, Gleason grade, CD44.

Introduction

Prostate cancer is one of the leading causes of death among male oncology patients [1, 2]. Despite efforts aimed at the early detection and radical treatment of prostate adenocarcinoma, the results remain unsatisfactory. After the initial response to treatment, prostate tumors relapse, usually in the more aggressive form of hormone-refractory/castration-resistant cancer [3, 4].

There is growing evidence supporting the hypothesis that prostate cancer cells originate from stem cells [5, 6]. To date, however, no study has proven the cancer stem cell (CSC) theory to be true in terms of prostate cancer development and progression [7]. Additionally, there is no agreement as to which markers are most sensitive to stem cells. Among the most often used markers are CD44, CD133 and ALDH1 [8-10].

In our preliminary assessment study that was performed on a smaller number of samples, CD44 expression was found to have a tendency to correlate with some of the clinical variables. Due to this reason, as well as to the fact that previously published studies on CD44 showed discrepancies between the obtained results, we decided to address these issues. We used CD44 as one of the most promising putative stem cell markers to evaluate the prevalence of prostate

cancer cells with stem cell-like features in prostate biopsies and in radical prostatectomy material. We tried to find a correlation between the expression of CD44 in both types of tissues and widely used prostate cancer prognostic factors such as Gleason grade, pathological stage according to TNM, the age of the patient and the preoperative plasma prostate-specific antigen (PSA) level. To the best of our knowledge, this is the first study in which this kind of approach has been used.

Material and methods

Formalin-fixed, paraffin-embedded (FFPE) tissue samples from 52 consecutive prostate biopsies and matched radical prostatectomy specimens from the years 2008 to 2011 were retrieved from the archives of the Pathology Department of the City Hospital in Poznan, Poland, or from a collection of cases sent for consultation in the private laboratory of one of the authors (JB). Only cases with accompanying basic clinical data were included in the study. Patient characteristics are shown in Table I.

Table I. Clinicopathologic findings of patients

Age, median (range)	63 (50-72)
PSA, median (ng/ml)	10.5
Gleason score (biopsies)	n (%)
4-6	28 (53.8)
7-8	24 (46.2)
Gleason score (prostatectomies)	n (%)
6	17 (32.7)
7-9	35 (67.3)
pT	n (%)
2a-2c	38 (73.1)
3a-3b	14 (26.9)

Sections were cut from all the prostate biopsy specimens containing adenocarcinoma, and the resulting slides were subsequently stained by immunohistochemistry, using a primary anti-CD44 antibody (monoclonal, MRQ-13, CellMarque, Rocklin, CA, USA) according to the manufacturer's protocol. In the case of radical prostatectomy material, two paraffin blocks containing the most representative Gleason pattern were used for the same procedure. At least 100 cancer cells (in core biopsies) or 500 cancer cells (in radical prostatectomy specimens) were counted using an OLYMPUS BX41 microscope. Only those cells with clearly visible membranous staining, at 10× magnification, were called positive for the expression of CD44. The percentage of cancer cells expressing CD44 was recorded and used in further analysis.

Statistical analysis was performed in Microsoft Office Excel 2007 using Spearman and Pearson correlations. A P value < 0.05 was considered significant.

Results

Forty-three prostate biopsies (82%) and 47 radical prostatectomy specimens (90%) contained CD44-positive cancer cells (Figs. 1 and 2). The main observation during the assessment of the expression of CD44 was heterogeneity in terms of the percentage of positively stained cancer cells, both in the prostate biopsies and in the radical prostatectomy material. The level of CD44 expression in prostate biopsies varied from 1% to 100% of cancer cells. However, in 31 cases (60%), the expression of CD44 did not exceed 30%. In radical prostatectomy specimens, CD44 expression was observed in 1% to 90% of cancer cells. As with the results seen in prostate biopsies, in the majority of radical prostatectomy cases (n = 33,

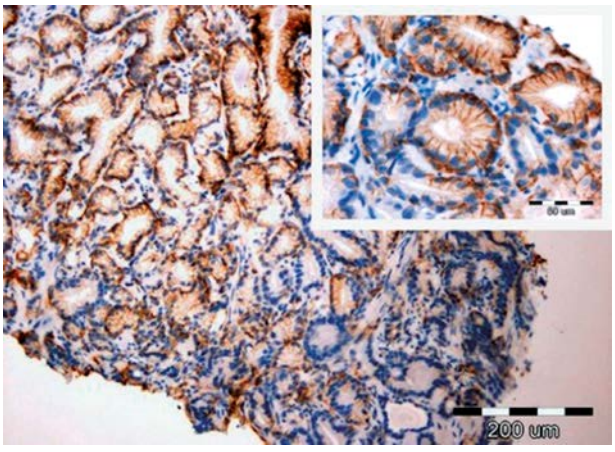

Fig. 1. Positive immunohistochemical reaction with anti-CD44 antibody in prostate biopsy (200×; inlet – 400×)

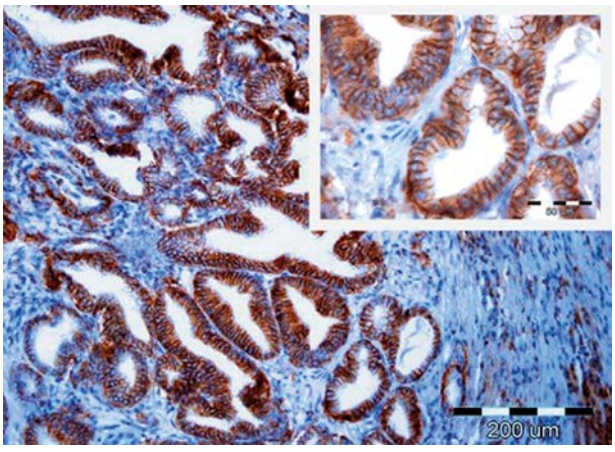

Fig. 2. Positive immunohistochemical reaction with anti-CD44 antibody in radical prostatectomy specimen (200×; inlet – 400×)

63%) the percentage of positively stained cancer cells did not exceed 30%. During the evaluation of immunohistochemistry slides, we observed that areas with higher differentiation (Gleason pattern 3) contained more positively stained cancer cells than parts of the tumor representing Gleason patterns 4 and 5. This observation was reflected in the statistical analysis, which showed a negative correlation between CD44 expression in cancer cells and the Gleason score, both in prostate biopsies and in radical prostatectomy specimens (Pearson –0.36 and –0.27, respectively) (Fig. 3). In matched tissue samples, we also found that higher CD44 expression in prostate biopsies correlates with a lower Gleason score in radical prostatectomy specimens (Pearson –0.30).

The comparison of CD44 expression between prostate biopsies and radical prostatectomy material showed a positive correlation between the percentage of positively stained cancer cells in both types of material (Pearson 0.37). When other clinical data were analyzed, such as patient age, preoperative serum PSA levels or tumor stage (pT), only the first parameter was found to correlate positively with CD44 expression in prostate biopsies (Pearson 0.33).

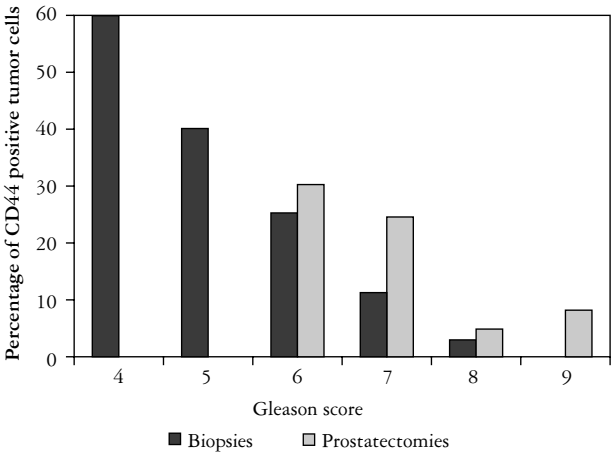

Fig. 3. Bar plot depicting negative correlation between Gleason score and percentage of CD44-positive tumor cells in prostate biopsies (in black) and radical prostatectomy specimens (in grey)

Discussion

The adhesion molecule CD44 has been identified as a stem cell marker in prostate cancer [6, 8, 11-14] (Table II). It is a transmembrane receptor for hyaluronan, which plays an important role in cell-to-cell and cell-to-matrix adhesion, migration, signaling, and tumor metastasis. There is growing evidence showing that CD44 is one of the most selective markers for cells presenting the features of stem cells. In his seminal work, Liu characterized prostate cancer cells in terms of CD44 expression [8]. Subsequently, Collins *et al.* studied the expression of three markers – CD44, α(1)β(2)integrin and CD133 – on cancer cells isolated from prostate tumors [6]. The results of their work and that of other researchers showed that cells expressing CD44 were capable of self-renewal and unlimited growth – characteristic features of stem cells [13, 15].

In one of the earliest studies, by Zhang *et al.*, performed on FFPE tissue, CD44 expression was evaluated in prostate cancer tissue samples obtained from 16 patients [16]. To date, there have been several other studies in which FFPE material was used to assess CD44 expression in prostate cancer tumors, the largest study being that of Kallakury *et al.*, which encompassed 106 patients [17]. The percentage of cases classified as positive, in terms of CD44 expression, was variable between the studies and ranged from 18% in the work by Eaton *et al.* up to 80% in the study by De Marzo *et al.* [18, 19]. This was probably due to differing, sometimes complicated criteria applied for the assessment of immunohistochemistry reactions. In our work, we tried to use the most objective rules by evaluating the percentage of cancer cells with clearly visible membranous staining at 10x magnification. This type of approach resulted in findings where 82% of prostate biopsies and 90% of radical prostatectomy specimens were found to con-

Table II. Previously published studies on CD44 expression in prostate cancer

Authors	No. of cases	No. of CD44 positive cases (%)	Correlation with Gleason score
Paradis *et al.*	38	14 (37)	no
Kallakury *et al.*	106	50 (47)	negative
De Marzo *et al.*	51	41 (80)	negative
Ugolkov *et al.*	65	47 (72)	no
Eaton *et al.*	11	2 (18)	no
Zhang *et al.*	16	8 (50)	positive*
Makarewicz *et al.*	43	37 (86)	no

**no statistical significance*

tain positively stained cancer cells. However, further analysis showed that in the majority of positive cases, from either specimen type, the percentage of CD44 expressing cancer cells did not exceed 30% (60% and 63% of cases, respectively).

The most important question we addressed in our study concerned the ability to predict tumor grade and stage on the basis of CD44 expression in the cancer cells of prostate biopsies. Since there is growing evidence to indicate that the level of CD44 expression may have an impact on response to adjuvant therapies, that is radio- and chemotherapies applied in several types of human cancers, awareness of CD44 expression levels could help to stratify prostate cancer patients into groups of different prognosis, depending on the probability of clinical response [20-23]. The data from our study showed a statistically significant negative correlation between the expression of CD44 in the cancer cells of prostate biopsies and Gleason score, assessed both in prostate biopsies and in tumor specimens from radical prostatectomy. Several other researchers have observed the same negative correlation in patients with prostate cancer after radical prostatectomy [17, 19, 24]. However, only one study, by Zhang *et al.*, reported a positive correlation between these two parameters, though the number of cases in their study was very low, comprising just 11 cases [16]. Other authors have found no correlation between CD44 expression and Gleason score [12, 15, 18, 25]. Since the methodology used in the cited papers is not consistent, it might be a source of the discrepancies and makes the direct comparison between different studies difficult.

The following question arises on the basis of our and aforementioned studies: is CD44 a marker of true CSCs, or is it only a marker of differentiation between normal and malignant cells? Since we and other groups have observed lost CD44 expression in prostate cancers with higher Gleason scores, indicating a lower level of cancer cell differentiation, CD44 could be treated as a differentiation marker only. Liu, however, found that CD44-expressing cancer cells from prostate cancer cell lines metastasize readily in animal models [8]. Liu also pointed out the ability of those cells to recognize the cells in high endothelial venules present in lymph nodes and bone marrow, a feature that allows them to form metastatic foci in such sites. Paradis *et al.* showed that the ability of CD44-expressing prostate cancer cells to invade the surrounding tissue and to metastasize could depend on the role of the CD44 molecule as a receptor for hyaluronan, an extracellular matrix component which may participate in the process of invasion [15]. Additionally, Tang *et al.* observed the inhibition of tumor growth and metastases in mice inoculated with prostate cancer cells from a cell culture treated with miRNA 34a, which blocks CD44 expression in these cells [26].

One possible explanation for these observations is that cancer cells expressing CD44 and other stem cell markers, such as ALDH1 and CD133, represent a sub-population of cells with stem-like features, and with high proliferative and metastatic potential. In the primary site, these cells give rise to primary low-grade prostate carcinomas, which subsequently dedifferentiate to high-grade cancer by losing CD44 expression. Meanwhile, stem cell like cancer cells expressing CD44 could invade the vascular system and metastasize to other organs. In two recent papers, researchers found loss of standard CD44 form (CD44s) expression during prostate cancer progression [27, 28]. This concept should be further evaluated in comprehensive animal models and in studies of stem cell markers' expression in primary and metastatic prostate cancers.

Conclusions

In conclusion, CD44, as a putative stem cell marker, plays an important role in prostate cancer development and progression. To the best of our knowledge, the present study showed for the first time that the level of CD44 expression in prostate biopsies correlates positively with its expression and negatively with the Gleason score in matched radical prostatectomy specimens from prostate cancer patients. Additionally, we confirm the results of other authors which show that the percentage of CD44-expressing cancer cells in prostate biopsies negatively correlates with the Gleason grade of tumors, both in prostate biopsies and in radical prostatectomy material. This information also provides a rationale for the recognition of CD44 as a surrogate marker of tumor differentiation. Taken together, these results imply that by assessing CD44 expression in prostate biopsies, one may predict the level of CD44 expression in radical prostatectomy specimens. This information could be very helpful for clinical oncologists, since there is growing evidence that CD44, together with other putative stem cell markers, plays an important role in predicting response to cancer therapy. The importance of our findings warrants further prospective studies.

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Address for correspondence

Konstanty Korski, MD, PhD
Department of Pathology
The Greater Poland Cancer Centre
Garbary 15
61-886 Poznan, Poland
e-mail: konstanty.korski@wco.pl