CD82 tumor suppressor expression in primary and secondary circulating prostate cells (CPCs) in blood in patients with prostate cancer

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ABSTRACT

Objective: To determine CD82, a metastasis inhibitor, expression in circulating prostate cells (CPCs) in blood before and after definitive treatment, its association with clinical parameters and the presence of bone micrometastasis.

Methods and patients: A cross-sectional multicentric study of consecutive prostate cancer patients attended to within the time frame of 2008 and 2009. Mononuclear cells were isolated from 8 mL of venous blood by differential centrifuge and CPCs were detected with anti-prostate specific antigen (PSA) and identified

Resumen

Objetivo: Determinar la expresión de CD82, un inhibidor de metástasis, en las células prostáticas en la circulación sanguínea antes y después del tratamiento definitivo, la asociación con los parámetros clínicos y la presencia de micrometástasis ósea.

Métodos y pacientes: Estudio transverso, multi-céntrico de pacientes consecutivos con cáncer prostático atendidos entre los años 2008 y 2009. Las células mononucleares fueron aisladas de 8 mL de sangre venosa por centrifugación diferencial y las células prostáticas primarias y secundarias, en la circulación sanguínea (CPCs) detectadas con anti-antígeno prostático específico (APE)
with immunocytochemistry. Positive samples were subclassified with anti-CD82. Details of stage, age and serum PSA level were registered. A bone marrow biopsy sample was processed in the same manner.

**Results:** A total of 105 patients participated; 30 pretreatment and 75 posttreatment. There was an association between CPC detection frequency, clinical stage and Gleason score. CD82 expression was associated with a low Gleason score and the absence of bone metastasis.

**Conclusions:** CD82 expression in CPCs is associated with low grade tumor and does not inhibit cancerous cell dissemination but it inhibits its implantation in bone marrow and is associated with local non-bone recurrence.

**Key words:** CD82, prostate cancer, micrometastasis, circulating tumor cells, Chile.

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**INTRODUCTION**

The majority of prostate cancer (CaP) patients present with localized disease at the time of diagnosis. However, 30-40% of these patients will develop biochemical recurrence after radical surgery therapy.

The detection of circulating prostate cells (CPCs) in blood at the moment of CaP diagnosis or during follow-up could signal patients with greater risk of developing micrometastasis who will probably need additional therapy to surgery.

There is a correlation between the presence of CPCs and established prognostic factors, clinical stage and Gleason score but not with serum prostate specific antigen (PSA) level. Similar results have been obtained through the study of isolated tumor cells (ITCs) found in bone marrow.

The original tumor is a mixture of different cell populations with different characteristics, some with the capacity to disseminate or proliferate. In the last decade the discovery of the so-called tumor suppressor genes has provided new evidence about the metastatic process. When these genes are expressed they inhibit the development of macro and micrometastasis without altering primary tumor growth. The products of these genes can reduce the activity of one or two steps in the metastatic process, causing inhibition in the dissemination, implantation or in the ability to form colonies in distant tissue.

The KAI1 tumor suppressor gene is located in the p11.2 region of chromosome 11, and codes for CD82, a glycoprotein of the tetraspanin family. In benign prostatic tissues, as in the hyperplasia that express CD82, this expression is increased in benign hyperplasia associated with low grade cancer as well as in CaP with a Gleason score of 3 and 4, but expression is reduced or absent in cases of high grade cancer and in cases of macrometastasis. It has been postulated that CD82 acts due to C-proteinase which plays a crucial role in the cellular cycle, in migration and in invasion.

In the present cross-sectional study double-immunomarking was used to detect CD82 coexpression in primary and secondary CPCs, the relation to clinical parameters and the absence or presence of micrometastasis in the bone marrow of CaP patients.
OBJECTIVE

The objective of the present study was to determine the expression of CD82, a metastasis inhibitor, in CPCs in blood before and after definitive treatment, clinical parameter association and the presence of bone micrometastasis.

MATERIALS AND METHODS

A prospective study was carried out on CaP patients in the time frame of January 2006 to August 2008 at an Oncology Center in Santiago de Chile. Age, total serum PSA at the moment of taking the sample, clinical stage, Gleason score and date of diagnosis were registered for each patient.

After obtaining a letter of informed consent from each patient a sample of 4 mL of venous blood from cubital vein was taken, using a 21G needle and a tube with EDTA as anticoagulant (BD-Vacutainer®).

Bone marrow biopsy was taken from the upper posterior iliac crest and three samples were prepared.

Mononuclear cell separation: these cells were separated by differential centrifuge with Histopaque 1,077® (Sigma-Aldrich) according to the manufacturer’s instructions. To prepare each smear (silanized slides, DAKO, USA) 25 µL of supernatant were used. Smears were air-dried for 24 hours and then fixed with 70% ethanol, 5% formalin and 25% saline solution, neutralized with phosphate buffered saline (PBS) pH 7.4 for 5 min and washed 3 times with PBS.

Immunocytochemistry: PSA monoclonal antibodies, clone 28A4 (Novocastra Laboratory, U.K.) were used in a concentration of 2.5 µg/mL to detect prostate cells. The reaction was developed with a system based on alkaline phosphatase- anti-alkaline phosphatase (LSAB2, DAKO, USA) with new fuchsin as well as chromogen and levamisole (DAKO, USA) as the endogenous alkaline phosphatase inhibitor. Manufacturer’s instructions were followed.

Positive PSA samples went through a second processing stage to detect CD82 clone 5B5 CD82 (Novocastra Laboratory, U.K.) with a 1:50 dilution. After incubation with peroxidase inhibitor (DAKO, USA), reaction was developed with a system based on peroxidase (LSAB2, DAKO, USA) with Vector VIP (Vector Laboratories, USA) as chromogen. Manufacturer’s instructions were followed.

The International Society for Hematotherapy and Graft Engineering (ISHAGE) criteria from 1999 were used to define CPC. CD82 expression was classified according to a semiquantified scale:

- 0 = no expression
- 1+ = part of the membrane expresses CD82
- 2+ = all the membrane weakly expresses CD82
- 3+ = all the membrane strongly expresses CD82

Patients were classified according to the percentage of positive PSA cells that expressed CD82: with >10% cells with CD82, 2+; plus, 3+ as positive. Micrometastasis was defined as fragments of bone marrow positive for PSA expressing cells (Image 1).

Statistical analysis: Descriptive statistics were used for the demographic variables, Student t test for the age differences and PSA and chi-square test was used for the differences in frequency of positive cells among the different subgroups. Alpha error was considered to be 0.05, beta error 0.20 and significance was considered when $P < 0.05$. Windows 98® Epi Info® program was used for the analysis.

Ethical considerations: The study was developed in accordance with the World Medical Association’s Declaration of Helsinki and was approved by the local Ethics Committee.

RESULTS

A total of 105 men presenting with CaP were included in the study. Of these patients 30 presented with recent diagnosis and 75 were in follow-up stage. Mean age was 71.3 ± 8.4 years, with 47-94 year range. Mean serum PSA level in the 30 patients prior to treatment was 6.81 ng/mL ± 4.45 ng/mL and in the 75 post radical prostatectomy follow-up cases was 0.41 ng/mL ± 0.39 ng/mL. CPCs in vein blood were detected in 64 patients (60.4%) with a mean 3.86 cells/mL ± 3.27 cells/mL; range of 1-15 cells/mL.

1. CPC detection:

According to stage: there was a correlation between the presence of CPCs and disease clinical stage. A total of 46.9% (23/49) of patients in stage two, 64% (34/50) in stage three and 100% (7/7) in stage four presented with CPCs.

There was significant statistical difference in CPC detection frequency between stage two and stage three patients ($P < 0.03$, two-tailed chi-square test) and between stage two and stage four patients ($P < 0.01$; two-tailed Fisher test).

There was no statistically significant difference between stage three and stage four patients ($P = 0.17$, two-tailed Fisher test), (Table 1).
There was statistically significant difference between pre- and posttreatment CPC detection frequency. Frequency in stage two patients was reduced from 13/18 pretreatment (72.2%) to 10/30 posttreatment (33%) (P <0.01, two-tailed chi-square test). There was no similar difference between stage two patients and stage three patients: 9/10 (90%) and 24/39 (61.5%), respectively; (P =0.13, two-tailed Fisher test). No statistically significant difference was found between stage two and stage three patients (13/18 vs 9/10, respectively; P = 0.38, two-tailed Fisher test). In contrast, there was statistically significant difference between stage two and stage three patients with regard to posttreatment frequency: 10/30 (33%) vs 24/39 (61.5%), respectively(P<0.038, two-tailed chi-square test). There was no statistically significant association between CPC detection frequency and total Gleason score between pre- and posttreatment patients. (Table 2).

2. CD82 immunomarking:
According to stage: Positive CPC detection for CD82 was more frequent in stage two than in stage three patients (P <0.005, two-tailed Fisher test); there was no statistically significant difference in the stage four comparison (Table 3).

According to Gleason score: CD82 expression in CPCs had an inverted relation with respect to Gleason score; CPCs detected in patients with a Gleason score ≥7 were negative for CD82. Mean CD82 expression value was significantly reduced with an increase in Gleason score (Table 4).

Association with the presence of bone micrometastasis: CD82 expression in patients with positive CPCs was associated with a lower bone micrometastasis frequency (P <0.005 two-tailed chi-square test), 5/17 vs 36/53, respectively (Table 5).
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Discussion

The present study is the first to demonstrate that CPCs express CD82 and the relation of that expression to Gleason score and clinical stage. Similar results were found in other studies that detected this marker in prostate tissue cells obtained from radical prostatectomy.10,11

The results of the present study suggest that CD82 expression is not enough in itself to inhibit CPC dissemination from primary tumor from distant tissue microfoci. Negative association with the presence of bone micrometastasis suggests that the function of CD82 is to inhibit bone implantation. Consequently the present authors postulate CD82- positive CPCs detected after radical therapy are manifested in local recurrence and could be treated with completely local therapy in contrast to CD82-negative CPCs that may arise from local or systemic recurrence and would need systemic therapy. The authors suggest that CPC presence implicates a higher risk of developing micrometastasis. CD82 coexpression is associated with low-grade tumors, a reduced risk of developing bone micrometastases and with patients with biochemical recurrence related to local recurrence. CPC detection and subclassification with CD82 could be a useful tool in follow-up of CaP patients for determining the necessity and type of complementary therapy.

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Bibliography


