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CD44 Variant Exons in Leukemia and Lymphoma

Elif AK•S•K,[1] Sevil BAVBEK,[2] Nejat DALAY[1]

[1]Departments of Basic Oncology, [2]Clinical Oncology, Oncology Institute, Istanbul University, Istanbul, Turkey

CD44 is a cell surface glycoprotein expressed on different cell types that functions in lymphocyte activation and homing, extracellular matrix adhesion and cellular migration. CD44 is encoded by a single gene composed of at least 20 exons. The standard CD44 protein (CD44S or CD44H) is the hematopoietic form of CD44 in lymphoid cells. Variant isoforms (designated from v1 to v10) are formed by addition of new exons to the extracellular domain. High levels of CD44v6 expression has been observed in some tumors and are associated with metastatic spread. The aim of the present study was to investigate and evaluate expression of the CD44v6 and v6-containing variants as a possible marker in chronic myeloid leukemia and lymphoma by reverse transcription-polymerase chain reaction. CD44 exon v6 was detected in all patients and all individuals in the control group. CD44v6-v10 mRNA was observed in 25 patients but in none of the subjects in the control group. CD44v6/v9-10, CD44v6-v7, CD44v6/v10 transcripts were detected in 11, 6, and 2 patients, respectively. CD44v6-7/v9-10 transcripts were not observed in either the patients or the healthy individuals. We conclude that CD44v6-v10 expression may be associated with hematologic malignancies. (Pathology Oncology Research Vol 8, No 1, 36–40, 2002)

Keywords: CD44, RT-PCR, leukemia, lymphoma

Introduction

CD44 is a membrane glycoprotein with diverse functions expressed in many cell types. The molecule is the surface receptor for hyaluronate.[4] It acts as the homing receptor for lymphocytes and is involved in extracellular matrix adhesion,[15,47] lymphocyte homing,[15] cellular migration[31] and metastasis.[25]

The human CD44 gene is located on the short arm of chromosome 11p13 and is composed of 20 exons.[3,45] Ten additional exons can be alternatively introduced into a common splice site in different combinations creating various splice variants designated as CD44v.[24,45] The standard form (commonly referred to CD44H or CD44S) of the molecule lacks all variant exons and is expressed on cells of hemopoietic and mesodermal origin.[23]

While the function of the hemopoietic form is well known, the function of the splice variants is less clear. Expression of specific variant exons has been shown in different tumors.[8,19,45] It has been suggested that splice variants with exon v6 could confer metastatic potential to cancer cells.[12] CD44v6 may play an active role in lymph node infiltration by tumor cells.[45] Expression of this exon has also been associated with worse prognosis.[20,28,39,43] However, there are reports ranging from correlation of the expression level with tumor progression[16] to downregulation of CD44v6 expression in tumors of certain cell types.[38] Thus, the contribution of the v6 variant to tumorigenesis is far from clear.

In this study we investigated the expression of the standard form, the specific CD44v6 variant and the v6-containing variants in hematological malignancies by reverse transcription polymerase chain reaction (RT-PCR) and compared the frequencies to a healthy control group.

Materials and Methods

Expression of CD44 and its exon v6 variants were investigated in peripheral blood of 28 patients (12 women and 16 men, mean age 47.7 ± 17.4) with lym-

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Correspondence: Dr Nejat DALAY, Department of Basic Oncology, Oncology Institute, 34390, Capa, Istanbul, Turkey. Tel: +90 212 531 31 00/184; Fax: +90 212 534 80 78¸ E-mail: ndalay@istanbul.edu.tr

Abbreviations: CML, chronic myeloid leukemia; HL, Hodgkin's lymphoma; NHL, Non-Hodgkin's lymphoma; RT-PCR, reverse transcription polymerase chain reaction.

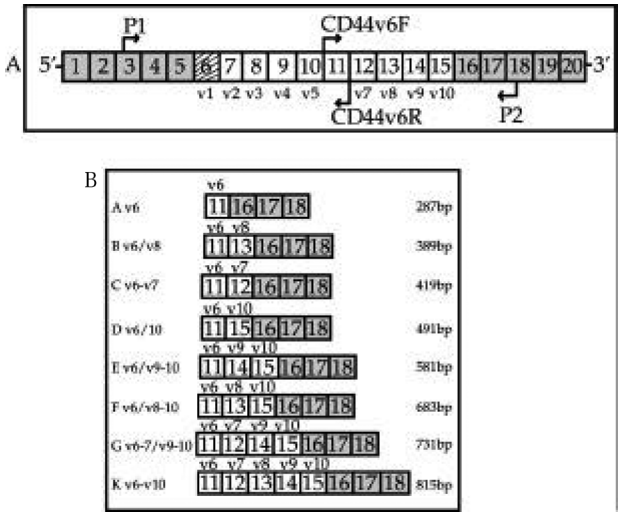

Figure 1. *(A) Schematic representation of the coding region of the human CD44 gene (grey boxes, constant exons; clear boxes, variant exons) and localization of the primers used to amplify CD44 transcripts. Exon 6 (stripped box) is not expressed in humans.[41] (B) Schematic representation of the different CD44 splice transcripts.*

phoma and 12 patients (5 women and 7 men, mean age 46.3 ± 16.9) with chronic myeloid leukemia. Among the patients with lymphoma 18 patients had Non-Hodgkin's lymphoma and 10 patients had Hodgkin's lymphoma. Peripheral blood of 22 healthy individuals (11 women and 11 men, mean age 30.4 ± 16.7) was used as the control group.

Peripheral blood leukocytes were isolated by Ficoll-Histopaque (Sigma Chemicals Inc., St Louis, USA) density gradient centrifugation. Total cellular RNA was prepared using 10^6 cells, followed by treatment with acid phenol/guanidinium isothiocyanate.[5] The amount of RNA was adjusted to 1 µg/µl spectrophometrically.

The Access RT-PCR System (Promega, Madison, WI, USA) was used for RT-PCR reactions. For amplification of CD44S primers P1 and P2 were used followed by nested PCR using the CD44v6 specific primer pair. These reactions for CD44S and CD44v6 yield PCR products of 482 bp and 129 bp, respectively.[3,26]

The sequence of the primers were:

P1 5'– GAC ACA TAT TGC TTC AAT GCT TCA GC -3'
P2 5'– GAT GCC AAG ATG ATC AGC CAT TCT GGA -3'
CD44v6F 5'– TCC AGG CAA CTC CTA -3'
CD44v6R 5'– CAG CTG TCC CTG TTG -3'

The location of the primers is depicted in *Figure 1a*.

For the detection of v6-containing variants the PCR reaction was performed using P2 and CD44v6F. The PCR products corresponding to the v6-containing variants ranged from 287 to 815 bp (*Figure 1b*).

Amplification was performed with 1 µg/µl of RNA in a total volume of 50 µl containing 5X Reaction Buffer, 1mM $MgSO_4$, 10 mM of each dNTP, 20 pmole of outer primers, 5U AMV Reverse Transcriptase and 5U Tfl DNA Polymerase. cDNA synthesis was performed at 48°C for 30 min and 94°C for 2 min. cDNA was subjected to 30 cycles of PCR at 94°C for 60 seconds, 55°C for 60 seconds and 72°C for 2 minutes.

1 µl of the first amplification product was amplified with the nested primers in a final volume of 50 µl containing 1X PCR Buffer (100mM Tris-HCl pH: 8.8, 200mM $(NH_4)_2SO_4$, 0.1% Tween 20), 2 mM $MgCl_2$, 10mM of each dNTP, 20 pmole of inner primers and 1U Taq polymerase. The reaction was initiated by denaturation for 2 minutes at 94°C and performed for 30 cycles at 94°C for 60 seconds, 55°C for 60 seconds and 72°C for 60 seconds with a final extension at 72°C for 5 min.

The PCR products were separated by electrophoresis in 2% agarose gels and visualised by staining with ethidium bromide. Hae III digested øX174 DNA and a 100 bp DNA marker (Promega, Madison, WI, USA) were used as size markers. Analysis of the bands was performed in a gel documentation system using the Bioprofil 1D Image Analysis Software (Vilber Lourmat, Marne-La-Valée Cedex, France).

Results

CD44v6 and exon v6-containing variants were investigated by RT-PCR in 40 patients and 22 healthy individuals. CD44v6 expression was detected in all patients and all individuals in the control group (*Figure 2*).

Among 40 patients with lymphoma and CML expression of CD44v6-v10 mRNA was observed in 25 (62.5 %) patients (*Figure 3*). 21 patients with lymphoma and 4 patients with CML expressed this variant. Interestingly, this variant was not observed in the control group. Thus, the difference was statistically significant ($p<0.001$).

CD44v6/v9-10, CD44v6-v7 and CD44v6/v10 transcripts were detected in 11 (27.5 %), 6 (15 %), and 2 (5 %) patients, respectively. Only 1 patient (2.5 %) was found positive for CD44v6/v8-10 mRNA expression. No CD44v6-7/v9-10 transcripts were detected either in the patients or in the healthy individuals. CD44v6/v8-10 and CD44v6-v10 transcripts were not detected in control group.

The frequencies of the v6-containing transcripts in the patients and the control group are summarized in *Table 1.*

The frequencies of the variants CD44v6-v7, CD44v6/v9-10 and CD44v6-v10 were higher in early stage, high or intermediate grade lymphoma and in the patients with metastasis. No correlation was found between the presence of variants CD44v6/v8 and CD44v6/v10 and any of the clinical parameters. Presence of the K variant was significantly associated with malignancy as well as with metastasis in lymphoma ($p<0.001$).

In patients with CML, variants C and E were detected in 3 patients (25 %) and variant K in 4 patients (33.3 %). Variants B, D and F were not observed in this group.

Discussion

Expression of CD44v isoforms in abnormal amounts and/or compositions may accompany malignant transformation.[25] It has been suggested that certain CD44 isoforms are upregulated and may play a role in the development of human tumors.[12,20,26,37,40] Expression of certain splice variants may also be restricted to tumors[26] and can promote the metastatic behaviour of cancer cells.[38] It has been suggested that increased expression of CD44v6 may represent a characteristic feature of human tumors[9] and act as a marker of tumor progression in human breast and colorectal cancer.[17,20,28,30,39]

Expression of the CD44 variant exons has been extensively investigated in colon[11,36,49] and to a lesser extent in lung,[10,29,46,48] breast[6,36,49] and gastric[7,14,27] cancers. However, although serum levels of the soluble CD44 has been investigated[32,33] reports on the expression of CD44 variants in hematological malignancies are rare. In Non-Hodgkin's lymphoma an upregulation of CD44 variants has been observed in aggressive tumors and correlated with an unfavorable clinical outcome.[35,44] Serum levels of circulating CD44 have been shown to correlate with the clinical treatment response in lymphoma.[32,33] Elevated levels of CD44v expression were noted in peripheral blood lymphocytes of patients with leukemia and lymphoma, multiple myeloma and polycythemia vera.[22]

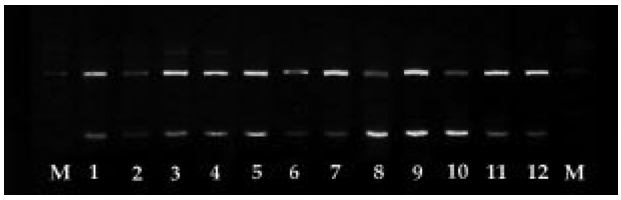

Figure 2. *RT-PCR detection of CD44S (482 bp) and CD44v6 (129 bp) transcripts by amplification with CD44S- and CD44v6-specific primers in the same reaction tube. Lanes 2,7: patients with non-Hodgkin's lymphoma, lanes 1,3,4,5,6: patients with Hodgkin's lymphoma, lanes 7-12: control group, lanes M: molecular weight marker.*

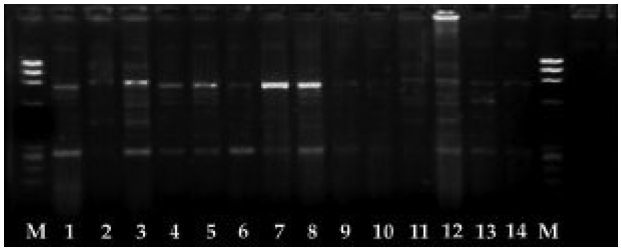

Figure 3. *Analysis of CD44v6-v10 variants expression. Lanes 4,7,8,9: patients with Hodgkin's lymphoma, lanes 5,6,14: patients with non-Hodgkin's lymphoma and lanes 1,2,3,10,11,12,13: patients with chronic myeloid leukmia. Lanes M: molecular weight marker.*

Table 1. **Incidence of various CD44 variants in the patients and the control group**

Variants	*Control group*	*Lymphoma*	*CML*	*All Patients*
B	9.1%	3.6%	–	2.5%
C	4.5%	10.7%	25%	15%
D	13.6%	7.1%	–	5%
E	4.5%	28.6%	25%	27.5%
F	–	10.7%	–	2.5%
G	–	–	–	–
K	–	75%	33.3%	62.5%

The aim of this study was to investigate and evaluate expression of CD44v6 and v6-containing variants in patients with lymphoma and CML using RT-PCR. In our study we detected CD44v6 expression in both the patient and control groups. This finding is in line with a previous study in which circulating CD44v6 has been detected in all controls.[34] Since CD44 variants are also present in activated normal human lymphocytes, expression of variant exons in healthy subjects is not unexpected.[23] CD44v6 is involved in B and T cell activation ***in vivo***[9,13,23] and it has been shown that lymphocytes can transiently express CD44v6 after they contact the antigen. Variant exons have also been reported in normal tissues.[1,9] Our results support the view that expression of exon v6 is an early event in the activation of lymphocytes.[18,41,42]

Presence of the C (CD44v6-v7), E (CD44v6,v9-10) and K (CD44v6-v10) variants in patients with lymphoma is in line with a previous study suggesting a significant role of the variant exons in tumor development.[2] A higher frequency of the v6-v10 variant in patients with metastasis supports the hypothesis that exon v6-containing isoforms may be associated with a metastatic phenotype.[9] Detection of the C, E and K variants is also in concordance with a report on patients with hematological malignancies.[22]

Since these isoforms occur in an environment of non-malignant cells it has been suggested that the ratio of the specific variant to the hematopoietic form may be important.[21] In this report the most striking difference was the presence of the v6-v10 variant in a significant proportion of the patients but none of the individuals in the control group. Thus, this particular splicing pattern may offer a novel molecular marker in the evaluation of patients with hematological malignancies.

In conclusion, our results indicate that CD44 isoforms involved in normal lymphocyte recirculation may also be important in migration of their malignant counterparts. These data suggest a role for the CD44v6-v10 variant in high grade and metastatic lymphomas.

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