Prostate-specific Antigen Expression in Neoplastic Human Myeloid Cell Lines, Ferdinando Mannello,1* Francesca Luchetti,2 Domenico Lanciali,3 Serafina Battistelli,1 Stefano Papa,2 and Giancarlo Gazzanelli1 (1 Instituto di Istologia ed Analisi di Laboratorio, Facoltà di Scienze, MFN Università, Via E. Zeppi, 61029 Urbino, Italy; 2 Instituto di Scienze Morfologiche, Università Studi, 61029 Urbino, Italy; 3 Laboratorio Analisi, Ospedale Civile, 61029 Urbino, Italy; * author for correspondence: fax 39-722-322370, e-mail mannello@bio.uniurb.it)

Prostate-specific antigen (PSA) is a 33-kDa serine protease produced by prostatic epithelial cells under the influence of the androgen receptor (1). Although PSA previously was thought to be a prostatic tissue-specific protein not expressed in any other tissue in men or women, at present it is considered to be present in many nonprostatic tissues expressed in any other tissue in men or women, at present was thought to be a prostatic tissue-specific protein not ever, it is steroid hormone-mediated pathological conditions is not organ- or sex-specific; however, it is steroid hormone-mediated (4). In fact, several cell lines (from breast, ovary, prostate, and lung cancer) showed specific PSA production modulated by steroid hormones (3, 5, 6). Recently, nested PCR has shown PSA messenger RNA in a variety of non-prostate cells, including hematological cell lines (7, 8), nondiseased peripheral blood (9, 10), and bone marrow specimens (11, 12). The finding of PSA expression within cells that are outside of the prostate is of great clinical utility in establishing the nonspecificity of PSA as an indicator of micrometastatic malignant disease (13–15).

To establish the presence of PSA immunoreactivity in nondiseased human peripheral blood unfractionated leukocytes (PBLs) and in leukemic cell lines, we undertook the present study on PSA content and immunoreactivity in blood samples from 10 healthy female and male volunteers, ages 25–42 years (mean, 33 ± 4 years). After collection, the blood samples were enriched in unfractionated mononuclear cells by centrifugation at 500g for 20 min at 4 °C on Ficoll-Hypaque (Pharmacia). Erythrocyte-depleted blood samples were prepared and counted after treatment with a lysing solution (Coulter Electronics). Sera from PSA-negative healthy control females were used as the negative control.

The leukemic cell lines HL-60 (promyelocytic), K562 (erythromyeloid), and U937 (premonocytic), all myeloplasma free, were maintained in RPMI supplemented with 100 mL/L heat-inactivated fetal calf serum (Irvine Scientific), 2 mmol/L glutamine, 100 000 units/L penicillin, and 100 mg/L streptomycin (Seromed, Biochrom KG) in a 5% CO2 humidified incubator at 37 °C. The prostate carcinoma cell line LNCaP was grown in RPMI medium supplemented with 50 g/L fetal calf serum in a separate CO2 humidified incubator at 37 °C to avoid the possibility of cross-contamination. This cell line is known to produce PSA and was used as the positive control. The cell pellets were lysed for 30 min on ice with 1 mL of lysis buffer containing 50 mmol/L Tris, pH 7.5, 150 mmol/L sodium chloride, 5 mmol/L EDTA, 10 g/L nonidet NP-40 surfactant, 1 mmol/L phenylmethylsulfonyl fluoride, and 1 mg/L each of aprotinin and leupeptin as proteinase inhibitors (16). The suspensions were frozen at −80 °C and thawed at 37 °C three times and sonicated for 5 cycles at 40 W of output for 30 s on ice (Heat-System Ultrasonic Inc.). Cell debris was pelleted at 9000g at 4 °C for 30 min, and the supernatants were collected and immediately assayed for PSA and total protein concentration.

The total protein content was determined with either the Coomassie G-250 or the bichinonic methods (with commercially available kits from Bio-Rad Laboratories and Pierce Chemical Co., respectively). Total and free PSA concentrations were measured by microparticle enzyme immunoassay (16, 17), using a monoclonal mouse anti-human PSA antibody (AxSYM® from Abbott Laboratories). The detection limits of the assay were 0.02 μg/L for total PSA and 0.01 μg/L for free PSA. To exclude the possibility of matrix artifacts, PBLs and leukemic cell extracts were serially diluted in PSA-negative female serum and reanalyzed for the response linearity. The analytical recovery of at least two concentrations of purified PSA added to the leukemic cellular extracts (3.5 and 7.0 μg/L) was tested (18). All the assays performed on the cellular extracts were carried out in triplicates of three in at least six independent experiments.

Reagents and equipment for Western blotting were purchased from Bio-Rad Laboratories. Our protocols were followed throughout, using the primary anti-human PSA monoclonal mouse antibody from Dako (16). Results, expressed as mean ± SD, were considered to be statistically significant when P < 0.01. All statistical analyses were performed with the StatView, Ver. 4.1, package (Abacus Concepts Inc.) on a Macintosh Power PC (Apple Computer).

The present work was carried out in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 1983.

The prostate cancer cell line LNCaP produces and secretes PSA in the culture medium; in our conditions, the
extract from 10^7 LNCaP cells showed a high total PSA content, of which ~95% was free (Table 1). The mean PSA concentration in extracts of PBLs from healthy subjects (n = 8) was 0.04 ± 0.02 μg/L, close to the limit of detection of the assay and below the biological limit of detection (0.06 μg/L) previously reported for this assay (19). However, total PSA content in unfractionated PBLs showed no significant difference between men and women, and it was not statistically different from the zero calibrator. On the other hand, the total PSA content in extracts from neoplastic myeloid cell lines was significantly greater than in nondiseased PBLs (P = 0.0058, P = 0.0008, and P = 0.0004 for the HL-60, K562, and U937 cell lines, respectively). The percentage of noncomplexed PSA detectable in leukemic cell extracts was 97%, 95%, and 93% for the HL-60, K562, and U937 cell lines, respectively.

The linearity studies revealed a good linear correlation between PSA concentration and dilution (r^2 = 0.97, r^2 = 0.99, and r^2 = 0.99 for the HL-60, K562, and U937 cell lines, respectively). The average analytical recovery of purified PSA added to cytosolic extracts from mononuclear leukemic cell lines was 97%, 95%, and 93% for the HL-60, K562, and U937 cell lines, respectively.

In all leukemic cell lines examined, Western blotting detected a major characteristic immunoreactive band of ~33 kDa of molecular mass, reflecting the specific presence of free PSA (Fig. 1).

A previous paper convincingly demonstrated that PSA messenger RNA is expressed in the HL-60 promyelocytic leukemic cell line, whereas the related serine protease is below the detection limit in cell lysates under the extraction conditions used (7). On the other hand, a recent paper showed that hematological cell lines (including HL-60) were negative for PSA mRNA, even if the frequency of positivity varied widely from line to line (8). Immunoreactivity for PSA in bone marrow extracts was recently reported (23); these data confirmed previous evidence (utilizing mRNA PCR method, immunohistochemistry, and antibody detection on blots) that PSA-positive cells may be present in the blood mononuclear cell fraction, in lymph nodes, and in bone marrow mononuclear cells from a variety of patient populations, including females (9, 11–14). However, this is a matter of a recent debate; in fact, other studies failed to detect PSA mRNA in the peripheral blood of benign prostatic patients and healthy subjects (10, 15, 24). To our knowledge, this is the first reported expression of total and free PSA in neoplastic myeloid cells obtained from different and well-established leukemic cell lines and detected by a commercial method. The increased PSA immunoreactivity in human neoplastic leukemic cell lines, compared with the nearly undetectable values from nondiseased unfractionated peripheral leukocytes, gives further evidence of the presence of this biochemical marker in non-prostate sources as a widely distributed serine protease. Although the biological effects and the physiologic role of PSA in leukemic cells are currently unknown, the presence of PSA messenger RNA and the active PSA protease in some cells of non-prostate origin might interfere with methods to detect micrometastases (7, 8, 11, 25, 26).

### Table 1. PSA and protein content in various cell lines.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Total Protein, mg/10^7 Cells</th>
<th>PSA, ng/10^7 Cells</th>
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</thead>
<tbody>
<tr>
<td>LNCaP (n = 6)</td>
<td>1.18 ± 0.19</td>
<td>26.20 ± 1.89</td>
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<tr>
<td>HL-60 (n = 6)</td>
<td>0.83 ± 0.24</td>
<td>0.43 ± 0.11</td>
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<tr>
<td>K562 (n = 6)</td>
<td>2.34 ± 0.42</td>
<td>0.77 ± 0.15</td>
</tr>
<tr>
<td>U937 (n = 6)</td>
<td>1.45 ± 0.33</td>
<td>0.37 ± 0.08</td>
</tr>
<tr>
<td>PBLs (n = 8)</td>
<td>0.75 ± 0.46</td>
<td>0.12 ± 0.09</td>
</tr>
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References
Dysfunctional Factor VII Variant (FVII Tondabayashi) with R79Q: Determination of Mutated Site with Monoclonal Anti-Human Factor VII Antibody (B101/B1), Osamu Takamiya17 and Shigeru Takeuchi1 (1 Department of Medical Technology, School of Allied Medical Sciences, Shinsu University, Matsumoto 390-0820, Japan; 2 Department of Pediatrics, Saiseikai Tondabayashi Hospital, Tondabayashi 584-0082, Japan; * author for correspondence: fax 81 236-37-2391, e-mail itosamu@gipac.shinshu-u.ac.jp)

Inherited factor VII (FVII) deficiency is a rare autosomal recessive disorder that occurs in roughly 1 out of 500,000 people. Although some patients with homozygous FVII deficiency have a life-long tendency to bleed, a coincident thromboembolic complication has been reported (1). In general, the bleeding tendency of FVII deficiency is not as severe as that of hemophilia. The clinical features are quite variable, with a rather poor correlation between measured coagulation activity and clinical bleeding tendency (2). In laboratory testing, FVII deficiency has been divided into three types: cross-reactive material (CRM)-type, with decreased synthesis of the FVII molecule; CRM+, with decreased FVII activity but an antigen concentration within reference values; and CRM−, with reduced synthesis of FVII (3). Specific FVII variants have been characterized as FVII molecules that give different procoagulant activities using tissue factor (TF) from various sources, such as human, rabbit, and bovine brain TF (4–6). Some of the dysfunctional FVII variants possess either an Arg 79-to-Gln substitution in the first epidermal growth factor (EGF)-like domain or an Arg 353-to-Gln substitution in the catalytic domain of FVII (7–11).

In the present study, using an ELISA with the monoclonal antibody that recognizes a specific epitope located in the three-dimensional structure near position 79 in the first EGF-like domain of human FVII (12), we determined the mutated site of a dysfunctional FVII variant that possessed different FVII activity with human placental TF than with rabbit and ox brain TF.

The propositus was a 12-year-old girl with FVII deficiency. She was born at 36 weeks gestation, had no hemorrhagic problems, and showed no evidence of abnormal hepatic function either at birth or later. Her parents were not consanguineous, and the family history did not reveal any bleeding disease. Decreased Normotest values were noted at her health examination at the age of 1 month. She was admitted to our Department of Pediatrics because of a suspected deficiency of vitamin K-dependent coagulation factors. The Normotest did not increase after the administration of vitamin K2 (3.5 mg). Hemostatic examination revealed a prolonged prothrombin time (Simplastin, Organon Teknika), whereas the bleeding time, platelet count, activated partial thromboplastin time, and fibrinogen were within reference ranges. The coagulation factor activities were within health-related reference values except for FVII, and her plasma showed no inhibitors of coagulation factors. However, the levels of FVII activity (FVII:c) exhibited different activation patterns when TFs from different sources were used. Her FVII:c was 7.2% of the healthy control when rabbit TF was used, and 40% and 65% when human placenta and bovine brain TF, respectively, were used. Abnormal coagulation values were observed constantly over a period of 10 years.

The FVII:c and FVII:ag levels of the propositus and her family members are shown in Table 1. The FVII:c level using recombinant human TF was similar to that using human placenta TF. Both the father and sister of the propositus showed different activation patterns with human TF and rabbit TF, although their FVII:c levels were not as low as that of the propositus. The mother’s activation pattern did not vary with the TFs used. The FVII:ag level of the propositus was only 5% of healthy controls when determined by ELISA-1 binding with hVII-B101/B1 as the solid phase, although it was in the lower limit of the health-related reference range when determined by ELISA-2 and ELISA-3, respectively. As measured by quantitative immunoelectrophoresis (QIEP) (13), the FVII:ag level was 64% of healthy controls. The ELISA/QIEP ratio was 0.078 in ELISA-1, 0.984 in ELISA-2, and 1.016 in ELISA-3. The FVII:ag levels of the father and sister were...