Asynchronous Growth of Prostate Cancer Is Reflected by Circulating Tumor Cells Delivered from Distinct, Even Small Foci, Harboring Loss of Heterozygosity of the PTEN Gene

Hartmut Schmidt,1,2 Gabriela DeAngelis,1,3 Elke Eltze,1,2 Iris Gockel,1,2 Axel Semjonow,1,4 and Burkhard Brandt1

1Prostate Center, University Clinic; 2Institutes of Pathology and Clinical Chemistry and Laboratory Medicine, and 3Clinical Chemistry and Laboratory Medicine, and 4Institute of Tumor Biology, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany

Abstract

The clinical value of prostate-specific antigen (PSA)–positive circulating tumor cells (CTCs) is still a matter of debate and it is also still unclear if these CTCs actually represent the primary tumor. Therefore, we isolated PSA-positive CTCs from the peripheral blood of patients suffering from multifocal cancers and did genetic profiling of each cancer focus by a multiplex PCR–based microsatellite analysis (D7S522, D8S522, NEFL, D10S541, D13S153, D16S400, D16S402, D16S422, and D17S855). In 17 of 20 prostate cancer cases, the loss of heterozygosity (LOH) pattern of the CTCs was identical with only one focus of the primary tumor. Moreover, in six cases, the LOH pattern suggested that smaller foci, down to 0.2 cm3, might deliver CTCs. Interestingly, the highest number of LOHs was observed at the marker D10S541 (85%), the PTEN gene, which was observed much less frequently in unifocal prostate cancer (48%). Furthermore, the infrequently occurring LOH in the BRCA1 gene (38%) was found in four of the five cases where a biochemical recurrence was seen within 3 years after prostatectomy. Therefore, the data might support the assumption that CTCs in prostate cancer are derived from distinct foci of a primary tumor. The size of the tumor focus is not related to the delivery of cells. Although the number of cases that were investigated in this study was small, it might be suggested that the LOH at distinct markers such as D10S541 and D17S855 represent the genes PTEN and BRCA1, which might be associated with the occurrence of CTCs in the peripheral blood of patients as well as an early biochemical recurrence. (Cancer Res 2006; 66(18): 8959-65)

Introduction

In the past, various studies have been done to elucidate the problem of measuring the degree of prostate tumor burden in the peripheral blood stream to create a molecular staging, to assess the metastatic risk. In particular, the reverse transcriptase-PCR (RT-PCR) amplification technique of prostate-specific expressed genes, such as prostate-specific antigen (PSA) and prostate-specific membrane antigen, has been one of the most hope-inspiring approaches to solve these problems. One conclusion of the finding of PSA-positive cells by assessing PSA mRNA in the peripheral blood of prostate cancer patients was that prostate cancer cells must be able to invade the human body without lymph node involvement. However, the clinical usefulness of these methods has been the subject of some controversy (1, 2) because a positive RT-PCR result before radical prostatectomy does not predict pathologic stage and early PSA recurrence. This might be caused by the heterogeneity of the primary tumor harboring cell clones of different genotypes detected (e.g., for p53 mutations that are very frequent in multifocal prostate tumors; ref. 3). Furthermore, data obtained on microdissected tissue resulted in the identification of areas in the primary tumor with different microsatellite alterations associated with a distinct histologic differentiation (4). With these results in mind, we investigated isolated PSA and cytokeratin-positive circulating epithelial cells from peripheral blood and evidently separated foci of multifocal prostate cancers.

Materials and Methods

Twenty patients with histologically proven multifocal prostate cancer were selected for genetic investigations of (a) epithelial blood-borne cell clusters isolated from peripheral blood and (b) different foci from the primary tumor. Prostate cancer was diagnosed on H&E-stained sections of prostate. A topographical map was created to visualize the tumor dimensions, shape, multifocality, and relation to capsule and surgical margins (see Fig. 1).

The pathologic evaluation showed 6 patients with organ-confined tumors (pT2a pN0), 10 with capsule penetration (pT3a pN0, n = 8; pT3a pN1, n = 2), and 3 with invasion in the seminal vesicle (pT3b pN1, n = 1). Gleason scores were 3+3 (n = 5), 3+4 (n = 8), 4+3 (n = 2), 4+4 (n = 2), 4+5 (n = 1), and 5+4 (n = 1). Hapel grading was IIa (n = 3), IIb (n = 13), IIIa (n = 2), and IIIb (n = 2; see Table 34-C).

For circulating epithelial cell enrichment, we used our previously described methods (5) that combined density gradient centrifugation with immunomagnetic separation using cytokeratin antibodies. Standardized 50-mL samples of fresh EDTA-anticoagulated blood derived from these patients were screened for circulating epithelial cells.

For further studies, we used the MACS Carcinoma Cell Enrichment Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) following the instructions of the manufacturer with minor modifications. The antibody immunomagnetic bead–tagged cells were bound in the column within the magnetic field of the magnet in the MACS stand. The negative, nonmagnetically stained cells passed through the column and were collected in a tube as the “negative fraction.” For further immunocytochemical investigations, cytospins were prepared by centrifuging 200 μL of the cell suspension onto a poly-L-lysine–coated glass slide in a cytocentrifuge (Hettich, Tuttingen, Germany). For identifying and characterizing the epithelial cell clusters, we used the following immunocytochemical staining techniques. The prostate-specific origin of the cells was determined by counterstaining with PSA-specific antibodies (DAKO, Hamburg Germany) decorated with secondary antibodies that were labeled by fluorescent dyes (Alexa 488 antirabbit,
Cytokeratin 7 and cytokeratin 8 were visualized by Alexa 594 goat anti-mouse antibodies (Molecular Probes). The DNA of the cell nuclei was also assessed with an intercalating dye (4',6-diamidino-2-phenylindole) in counterstained fashion. Isotype controls were done to show the specificity of the antibody staining. As a positive control, cytospins of cell cultured LNCaP (clone FGC, ATCC no. CRL-1740) were used for all immunocytochemical studies. For a negative control, the negative, nonmagnetically stained cells that passed the MS column were used. The cytospins were screened with an Olympus BX-61 fluorescence microscope. Documentation and data analysis were carried out with the Analysis software (SYS, Münster, Germany).

**DNA isolation.** For comparative genetic investigation of the circulating PSA cell clusters, primary tumors, and blood of the patients, three different DNA isolation protocols were used. DNA isolation from peripheral blood was done with the QIAamp Blood Kit (Qiagen, Düsseldorf, Germany) and from formalin-fixed primary tumor tissue with the QIAamp DNA Mini Kit (Qiagen) following the instructions of the manufacturer.

On an inverse fluorescent light microscope (Leitz Diavert, Wetzlar, Germany), the fluorescent cytokeratin and PSA-positive clusters were detected and then fine-needle microdissected. The genomic DNA of these cells was isolated using a short protocol. First, the microdissected cells and clusters were incubated in 100 µL of low Tris-EDTA buffer (50 mmol/L Tris, 1 mmol/L EDTA, pH 8) containing 10 µL of proteinase K from the QIAamp DNA Mini Kit in a heating block at 56°C overnight. After cooling, ethanol (99.8%) was added to the sample to reach a final concentration of 70%. Precipitation was carried out by centrifugation in a Heraeus microcentrifuge at 14,000 rpm for 20 minutes. The supernatant was discarded, dried, and the DNA was eluted in low Tris-EDTA buffer.

<table>
<thead>
<tr>
<th>Primer locus</th>
<th>Primer sequences</th>
<th>Cytogenetic localization</th>
<th>Expected DNA fragment lengths (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7S522</td>
<td>5'-Fam-GCAGGACATGAGATGACTGA-3'</td>
<td>7q31.1</td>
<td>116-126</td>
</tr>
<tr>
<td></td>
<td>5'-GTTATGCCACTCCCTCACAC-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D8S258</td>
<td>5'-Fam-AGCTGCCAGGAATCAAAGGAGGAGG-3'</td>
<td>8p22</td>
<td>216-230</td>
</tr>
<tr>
<td></td>
<td>5'-GATGCTACATAAAAAGGAGGAGG-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5'-Fam-CCACAACTTCAGTGATGCC-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5'-GAGCTGCTTAAACATAGGG-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D10S541 (PTEN)</td>
<td>5'-Fam-CACACAGACACTCTCACACC-3'</td>
<td>10q23</td>
<td>153-175</td>
</tr>
<tr>
<td></td>
<td>5'-CCAGTAGAAGTTCAGGAGG-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D13S153 (Rb1)</td>
<td>5'-Fam-AGGGTGAAGTAAACCCCACTCC-3'</td>
<td>13q14.2</td>
<td>170-190</td>
</tr>
<tr>
<td></td>
<td>5'-Fam-GTCTAAGGCCCTCGAGTTGGG-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D16S400</td>
<td>5'-Fam-GTTTCACATTGGCACAGTAT-3'</td>
<td>16q22.2-23.1</td>
<td>165-179</td>
</tr>
<tr>
<td></td>
<td>5'-GAACCCTCCATGCTGACATT-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D16S402</td>
<td>5'-Fam-CTACCCATGCACAGAATC-3'</td>
<td>16q24.2</td>
<td>110-120</td>
</tr>
<tr>
<td></td>
<td>5'-CAAAGCAACACAGACTAAT-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D16S422</td>
<td>5'-Joe-GAGAGGAAGGTTGAAATACA-3'</td>
<td>16q24.2</td>
<td>105-129</td>
</tr>
<tr>
<td></td>
<td>5'-GTTTAAGCAGAATGGAATAT-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D17S855 (BCA1)</td>
<td>5'-Fam-GGATGGCGCCTTCTTAGAGG-3'</td>
<td>17q21</td>
<td>139-153</td>
</tr>
<tr>
<td></td>
<td>5'-ACACAGACTTTGTCCACTGCG-3'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PCR-based multiplex-microsatellite analysis. PCR amplification was done with AmpliTaq DNA polymerase (Appella, Darmstadt, Germany) in a 10-µL reaction volume for the microdissected cells and in a 25-µL volume for tumor- and blood-derived samples. The PCR reaction mix contained 200 nmol/L of each primer, 1 x GeneAmp buffer II, 2 mmol/L MgCl₂, 100 µmol/L of each GeneAmp deoxy nucleotide triphosphate (Appella), and 1 to 10 ng of sample DNA. Nine microsatellite markers on chromosomes 7q, 8p, 10q, 13q, 16q, and 17q were tested. The primer sequences for multiplexed microsatellite analyses are given in Table 1. The microsatellite markers were grouped in three multiplex PCRs of three markers each with the following variations of the primer concentration: multiplex-PCR no. 1, D7S522, NEFL, D13S153, and D17S855; multiplex-PCR no. 2, D8S258, and D16S400; multiplex-PCR no. 2, NEFL, D13S153, and D17S855; and multiplex-PCR no. 3, D10S541, D16S402, and D16S422.

Multiplex 1 and multiplex 2 worked with 0.3 µmol/L of each downstream and upstream primer whereas multiplex 3 showed a reliable performance only with 0.2 µmol/L of both D10S541 primers, 0.3 µmol/L of both D16S402, and 0.5 µmol/L of both D16S422 primers. Forward primers were labeled with the fluorescent dye Fam or, in the case of D16S422, with Joe.

PCR amplification was carried out with the GeneAmp System 9700, PE Applied Biosystems, Weiterstadt, Germany. A first DNA denaturation and polymerase activating step at 95°C for 10 minutes was followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, primer extension at 72°C for 30 seconds, and a final extension step at 72°C for 7 minutes, followed by a 4°C cooling step.

One to two microliters of the amplified PCR products were diluted in 20 µL of water (high-performance liquid chromatography grade) containing 0.5 µL of GeneScan 500 ROX fluorescent size standard (PE Applied Biosystems). The mix was denatured at 95°C for 2 minutes and cooled for at least 10 minutes at 4°C, followed by capillary electrophoresis on an ABI Prism 3700 DNA Analyzer (PE Applied Biosystems). Data analyses were carried out with the GeneScan Analysis Software 3.7.

We had tested the reproducibility of microsatellite PCR depending on the DNA concentration using serial dilutions (10, 4, 1, and 0.4 ng per reaction mix). This is shown for the marker D8S258 of DNA from tumor and blood samples (n = 10) by the means of the ratios of peak areas and SDs (see Fig. 2).

The allelic imbalance score was calculated in a semiautomated manner. This represents the ratio of the intensities of the two alleles in the tumor tissue in comparison with those occurring in nontumor tissue. The allelic imbalance score estimates the genetic imbalance of the two allelic intensities in the tumor. The reliability of peak area and lengths for allelic imbalance score determination had been tested earlier using 10 runs of independent leukocyte PCR product showing an SD of the quotient of the allele peak areas (a₁ / a₂) of 7%, resulting in cutoff values of 0.79 for the loss of the longer allele and 1.27 for the loss of the shorter allele as described elsewhere (6).

### Results

We investigated multifocal prostate tumors and PSA-positive CTCs in the peripheral blood of 20 patients by multiplex PCR. From those, 55 to 5,400 CTCs were isolated from 50 mL of blood. Nine polymorphic microsatellite markers were used for the comparison of the loss of heterozygosity (LOH) pattern from the different tumor foci and circulating tumor cell (CTC) clusters. The reproducibility of microsatellite PCR was shown with DNA from tumor and blood samples (n = 10) by the means of the ratios of peak areas and SDs (see Fig. 2). As shown in Table 2, heterozygosity ranged from 85% (D13S153 and D16S422) to 35% (NEFL). The highest numbers of LOH in the primary tumors and CTCs were shown for the marker D10S541 (92% and 85%, respectively), followed by D13S153 (82% and 76%) and D16S422 (86% and 71%). D17S855 (BRCAL, 39% and 39%) showed the lowest LOH frequency. However, four of the five cases with a biochemical recurrence within 3 years revealed a D17S855 LOH in the CTC and primary tumors. The highest frequency of LOH was seen in foci that were not identical in their genomic pattern to the CTCs. As such, for D8S258, the difference in the number of LOHs between nonspreading foci in the primary tumor and the CTCs was 29% (79% versus 50%). Only in one case did both of the two foci of the primary tumor show identical LOH compared with that of the CTCs. In one instance, neither of the two tumor foci showed an identical alteration pattern with the CTC. The investigated prostate cancer foci in 9 cases were of approximately the same size, whereas in 11 cases they were of very different size. Of those, in six cases the smaller foci (Table 3A) and in five cases the larger tumor foci (Table 3B) had an identical LOH pattern with the CTCs. The smallest foci only had a volume of ~0.2 cm³. A correlation between the numbers of CTCs and the t-PSA level, tumor staging, and differentiation could not be found (data not shown). Biochemical recurrence only occurred in this study in cases with lower numbers of CTCs (55-750 CTCs/50 mL; mean, 330 CTCs/50 mL; Table 3).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Informative cases</th>
<th>Al detected in primary tumor</th>
<th>Al detected in CTCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7S522</td>
<td>8 (40%)</td>
<td>6 (75%)</td>
<td>6 (75%)</td>
</tr>
<tr>
<td>D8S258</td>
<td>14 (70%)</td>
<td>11 (79%)</td>
<td>7 (50%)</td>
</tr>
<tr>
<td>NEFL</td>
<td>7 (33%)</td>
<td>6 (86%)</td>
<td>6 (86%)</td>
</tr>
<tr>
<td>D10S541</td>
<td>13 (65%)</td>
<td>12 (92%)</td>
<td>11 (85%)</td>
</tr>
<tr>
<td>D13S153</td>
<td>17 (85%)</td>
<td>14 (82%)</td>
<td>13 (77%)</td>
</tr>
<tr>
<td>D16S400</td>
<td>12 (60%)</td>
<td>9 (75%)</td>
<td>7 (58%)</td>
</tr>
<tr>
<td>D16S402</td>
<td>17 (83%)</td>
<td>14 (82%)</td>
<td>11 (63%)</td>
</tr>
<tr>
<td>D16S422</td>
<td>14 (70%)</td>
<td>12 (86%)</td>
<td>10 (71%)</td>
</tr>
<tr>
<td>D17S855</td>
<td>13 (65%)</td>
<td>5 (39%)</td>
<td>5 (39%)</td>
</tr>
</tbody>
</table>

Abbreviation: Al, allelic imbalance.

Table 2. Degree of heterozygosity and distribution of allelic imbalance in the blood-borne PSA-positive cell clusters and the two foci of the primary tumors from 20 prostate cancer patients

Figure 2. Reproducibility of the determination of an LOH by microsatellite PCR for decreasing DNA concentrations. Columns, mean of the ratios of peak areas (n = 10); bars, SD.
# Table 3. Number of PSA- and cytokeratin-positive cell clusters found in 50-mL blood of prostate carcinoma patients in comparison with staging, grading, Gleason, prostate volume, tumor volume, their total PSA level, biochemical recurrence, and a comparison the detected genetic aberrations of the different foci

<table>
<thead>
<tr>
<th>Patients</th>
<th>CTCs in 50-mL blood</th>
<th>Staging (Helpap)</th>
<th>Grading</th>
<th>Gleason</th>
<th>Prostate volume (cm$^3$)</th>
<th>PSA preoperative</th>
<th>Biochemical recurrence</th>
<th>Tumor volume (cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) CTC spread from smaller focus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (39) 500</td>
<td>pT$<em>{2c}$ pN$</em>{0}$</td>
<td>Ila</td>
<td>3 + 4</td>
<td>37</td>
<td>9.9</td>
<td>No</td>
<td>CTCs Focus 1 (3)</td>
<td></td>
</tr>
<tr>
<td>6 (97) 700</td>
<td>pT$<em>{2c}$ pN$</em>{0}$</td>
<td>IIb</td>
<td>4 + 3</td>
<td>26</td>
<td>9.1</td>
<td>No</td>
<td>CTCs Focus 1 (2)</td>
<td></td>
</tr>
<tr>
<td>8 (53) 200</td>
<td>pT$<em>{3b}$ pN$</em>{0}$</td>
<td>IIb</td>
<td>3 + 4</td>
<td>25</td>
<td>5.0</td>
<td>Yes (8 mo)</td>
<td>CTCs</td>
<td></td>
</tr>
<tr>
<td>10 (93) 700</td>
<td>pT$<em>{2c}$ pN$</em>{0}$</td>
<td>IIb</td>
<td>4 + 4</td>
<td>50</td>
<td>15.83</td>
<td>No</td>
<td>CTCs Focus 1 (5)</td>
<td></td>
</tr>
<tr>
<td>15 (33) 400</td>
<td>pT$<em>{3a}$ pN$</em>{0}$</td>
<td>IIb</td>
<td>3 + 4</td>
<td>41</td>
<td>9.5</td>
<td>No</td>
<td>CTCs Focus 1 (10)</td>
<td></td>
</tr>
<tr>
<td>17 (32) 750</td>
<td>pT$<em>{3a}$ pN$</em>{0}$</td>
<td>IIb</td>
<td>3 + 3</td>
<td>42</td>
<td>6.37</td>
<td>Yes (23 mo)</td>
<td>CTCs Focus 1 (1.8)</td>
<td></td>
</tr>
<tr>
<td>(B) CTC spread from larger focus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (85) 1,000</td>
<td>pT$<em>{3a}$ pN$</em>{0}$</td>
<td>IIb</td>
<td>3 + 4</td>
<td>43</td>
<td>9.45</td>
<td>No</td>
<td>CTCs Focus 1 (14)</td>
<td></td>
</tr>
<tr>
<td>3 (50) 55</td>
<td>pT$<em>{3a}pN</em>{1}$</td>
<td>IIIb</td>
<td>5 + 4</td>
<td>26</td>
<td>10.6</td>
<td>Yes (28 mo)</td>
<td>CTCs Focus 1 (2)</td>
<td></td>
</tr>
<tr>
<td>5 (117) 1,930</td>
<td>pT$<em>{3a}$ pN$</em>{0}$</td>
<td>IIb</td>
<td>3 + 4</td>
<td>32</td>
<td>22.63</td>
<td>No</td>
<td>CTCs Focus 1 (2)</td>
<td></td>
</tr>
<tr>
<td>7 (34) 875</td>
<td>pT$<em>{3a}$ pN$</em>{0}$</td>
<td>IIb</td>
<td>3 + 4</td>
<td>52</td>
<td>17.2</td>
<td>No</td>
<td>CTCs Focus 1 (5)</td>
<td></td>
</tr>
<tr>
<td>16 (112) 5,400</td>
<td>pT$<em>{3a}$ pN$</em>{0}$</td>
<td>IIb</td>
<td>3 + 3</td>
<td>35</td>
<td>12.51</td>
<td>No</td>
<td>CTCs Focus 1 (0.5)</td>
<td></td>
</tr>
<tr>
<td>(C) No difference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (50) 55</td>
<td>pT$<em>{3a}pN</em>{1}$</td>
<td>IIIb</td>
<td>5 + 4</td>
<td>26</td>
<td>10.6</td>
<td>Yes (28 mo)</td>
<td>CTCs Focus 1 (2)</td>
<td></td>
</tr>
<tr>
<td>9 (89) 700</td>
<td>pT$<em>{2c}$ pN$</em>{0}$</td>
<td>IIb</td>
<td>3 + 4</td>
<td>163</td>
<td>19.36</td>
<td>No</td>
<td>CTCs Focus 1 (3)</td>
<td></td>
</tr>
<tr>
<td>11 (116) 1,485</td>
<td>pT$<em>{2c}$ pN$</em>{0}$</td>
<td>IIa</td>
<td>3 + 3</td>
<td>37</td>
<td>6.34</td>
<td>No</td>
<td>CTCs Focus 1 (1)</td>
<td></td>
</tr>
<tr>
<td>13 (102) 3,900</td>
<td>pT$<em>{2c}$ pN$</em>{0}$</td>
<td>IIb</td>
<td>3 + 4</td>
<td>33</td>
<td>9.42</td>
<td>No</td>
<td>CTCs Focus 1 (3.5)</td>
<td></td>
</tr>
<tr>
<td>14 (111) 600</td>
<td>pT$<em>{3a}$ pN$</em>{0}$</td>
<td>IIb</td>
<td>4 + 4</td>
<td>65</td>
<td>14.79</td>
<td>Yes (7 mo)</td>
<td>CTCs Focus 1 (2.5)</td>
<td></td>
</tr>
</tbody>
</table>

(Continued on the following page)
Table 3. Number of PSA- and cytokeratin-positive cell clusters found in 50-mL blood of prostate carcinoma patients in comparison with staging, grading, Gleason, prostate volume, tumor volume, their total PSA level, biochemical recurrence, and a comparison the detected genetic aberrations of the different foci (Cont’d)

<table>
<thead>
<tr>
<th>Microsatellite markers</th>
<th>D7S522</th>
<th>D16S400</th>
<th>D8S258</th>
<th>D17S855</th>
<th>NEFL</th>
<th>D13S153</th>
<th>D16S402</th>
<th>D16S422</th>
<th>D10S541</th>
</tr>
</thead>
<tbody>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

(Continued on the following page)
Discussion

Prostate cancer is a heterogeneous disease, particularly in its multifocality, which has been reported in 50% to 76% of all cases of radical retropubic prostatectomy specimens. However, the clinical and prognostic significance of this finding is still unclear. Different foci of prostate carcinoma can differ in their morphologic growth pattern, suggesting that separated foci of a given prostate carcinoma might also have a different tumor biology (7). Primary tumors show a rather heterogeneous expression of proliferation-associated markers, such as Ki-67, and apoptosis-induced DNA fragmentation, showing the asynchronous growth of a prostate tumor (8). Microsatellite-based studies on microdissected tissue resulted in the identification of areas in the primary tumor with different alterations associated with a distinct histologic differentiation (3, 4, 9).

From RT-PCR–based studies, we know that CTCs must be able to invade the human body without lymph node involvement (1, 2, 10). We focused our work on polymorphic DNA sequences to show the prostate cancer origin of CTCs through detection of the same mutation in the primary tumor focus and the cells. We initiated our comparative multiplexed microsatellite PCR–based genetic studies between CTCs and separated foci of multifocal prostate cancers. We focused our attention on microsatellite markers at six different chromosomal regions that show a high frequency of heterozygosity and are frequently deleted in prostate cancer as is known from various microsatellite and comparative genomic hybridization studies. The marker D7S522 on 7q31.1 (11) is associated with tumor progression and is colocalized with caveolin-1. One of the most frequently deleted chromosomal regions in prostate carcinoma is localized at 8p21-22, and this deletion is assumed to be an early event in prostate cancer progression (12, 13). The marker D10S541 is colocalized with the well-known tumor suppressor gene PTEN (13). The marker D13S153 is closely related to the tumor suppressor gene RB1 (14). Loss of 13q14 is frequently observed in prostate cancer and is also associated with prostate cancer progression (14). In prostate carcinoma, loss of 16q22-24 is correlated with a higher tumor grade and metastatic disease (15, 16). The marker D17S855 is located within the tumor suppressor gene BRCA1 on 17q21 (17). A comparative genetic microsatellite-based study from Cheng et al. (3) on 8p12-22 and 17q21, looking at synchronous primary prostate tumors and lymph node metastasis, showed that discordant LOH patterns were present in 42% of all investigated cases. These results suggest that different foci within a tumor have a different potential to form lymph node metastasis, and it may also reflect the heterogeneity of the primary prostate cancer. Cheng et al. (3) assumed in these cases that the metastatic tumor cells were, in fact, derived from separate foci that were not sampled. Similarly to these results, our study shows that the CTCs were most likely derived only from one distinct focus and that other foci from the same tumor sometimes have additional LOH. Only in one of the investigated cases did both foci show identical LOH patterns, whereas in one case we were not able to identify the dispersing focus in the primary tumor. Interestingly, Qian et al. (18) were able to show by fluorescence in situ hybridization that small foci of the primary tumor can metastasize and that usually one or more foci shared the same chromosomal aberrations with the corresponding metastases. Indeed, here we were able to show in six cases that, in multifocal prostate cancer, a small focus, of only 0.2 cm^3 in size, may have been the more likely culprit to have delivered the CTCs than the larger focus.

Table 3. Number of PSA- and cytokeratin-positive cell clusters found in 50-mL blood of prostate carcinoma patients in comparison with staging, grading, Gleason, prostate volume, tumor volume, their total PSA level, biochemical recurrence, and a comparison the detected genetic aberrations of the different foci (Cont’d)

<table>
<thead>
<tr>
<th>Patients</th>
<th>CCs in 50-mL blood</th>
<th>Staging</th>
<th>Grading (Helpap)</th>
<th>Gleason</th>
<th>Prostate volume (cm^3)</th>
<th>PSA preoperative</th>
<th>Biochemical recurrence</th>
<th>Tumor volume (cm^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 (30)</td>
<td>780 pT3a pN1</td>
<td>IIIb</td>
<td>4 + 5</td>
<td>65</td>
<td>5.0</td>
<td>No</td>
<td>CTCs</td>
<td>Focus 1 (20)</td>
</tr>
<tr>
<td>12 (70)</td>
<td>2,000 pT4a pN0</td>
<td>IIIa</td>
<td>4 + 3</td>
<td>37</td>
<td>13.15</td>
<td>No</td>
<td>CTCs</td>
<td>Focus 1 (3)</td>
</tr>
<tr>
<td>18 (47)</td>
<td>500 pT3a pN1</td>
<td>IIIb</td>
<td>3 + 3</td>
<td>56</td>
<td>25.2</td>
<td>No</td>
<td>CTCs</td>
<td>Focus 1 (15)</td>
</tr>
<tr>
<td>20 (23)</td>
<td>120 pT3a pN1</td>
<td>IIIa</td>
<td>4 + 4</td>
<td>44</td>
<td>7.21</td>
<td>Yes (33 mo)</td>
<td>CTCs</td>
<td>Focus 1 (9.5)</td>
</tr>
</tbody>
</table>

NOTE: CC, cell clusters; A1, allele 1; A2, allele 2. ●, LOH; ○, no LOH; ○, uninformative.
Table 3. Number of PSA- and cytokeratin-positive cell clusters found in 50-mL blood of prostate carcinoma patients in comparison with staging, grading, Gleason, prostate volume, tumor volume, their total PSA level, biochemical recurrence, and a comparison the detected genetic aberrations of the different foci (Cont’d)

<table>
<thead>
<tr>
<th>Microsatellite markers</th>
<th>D7S522</th>
<th>D16S400</th>
<th>D8S258</th>
<th>D17S855</th>
<th>NEFL</th>
<th>D13S153</th>
<th>D16S402</th>
<th>D16S422</th>
<th>D10S541</th>
</tr>
</thead>
<tbody>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

References


Asynchronous Growth of Prostate Cancer Is Reflected by Circulating Tumor Cells Delivered from Distinct, Even Small Foci, Harboring Loss of Heterozygosity of the PTEN Gene

Hartmut Schmidt, Gabriela DeAngelis, Elke Eltze, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/66/18/8959

Cited articles
This article cites 18 articles, 9 of which you can access for free at:
http://cancerres.aacrjournals.org/content/66/18/8959.full.html#ref-list-1

Citing articles
This article has been cited by 12 HighWire-hosted articles. Access the articles at:
/content/66/18/8959.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.