

Detection of circulating tumor cells in the peripheral blood of patients with androgen-independent, advanced or metastatic prostate cancer

To the Editor: The presence of circulating tumor cells (CTC) in the peripheral blood of cancer patients has attracted a great deal of attention among investigators. The clinical significance of CTC has probably best been established in breast cancer, where it has been shown that the detection of CTC in metastatic patients correlates with early clinical relapse and patient death [1]. Gene expression profiling of CTC has been extended to other metastatic cancers including colorectal and prostate cancer [2,3].

Recently, a porous barrier density-gradient centrifugation system was developed that allows for enrichment of CTC from peripheral blood [4]. When coupled with the use of specific genes for real-time reverse transcriptase (RT)-polymerase chain reaction (PCR), the buoyant density separation approach

Research Center (GCRC) at the MUSC. The main objective of the study was to measure, by real-time, reverse transcriptase (RT)-polymerase chain reaction (PCR), the expression of a specific set of genes (EpCAM1, EpCAM2, XAG, S100P, DAG1, Claudin7, Ese1, Mal2, Spint2, ERG, and ETV1) in the peripheral blood of enrolled patients.

Results from 11 out of 15 enrolled patients could be analyzed and we found evidence of CTC in 8 of 11 subjects (6 of 8 for African-Americans). EpCAM1 and EpCAM2 were the most informative markers (Fig. 1). To the best of our knowledge, this is the first report identifying EpCAM2 as a marker for CTC in PCA, especially for African-American men.

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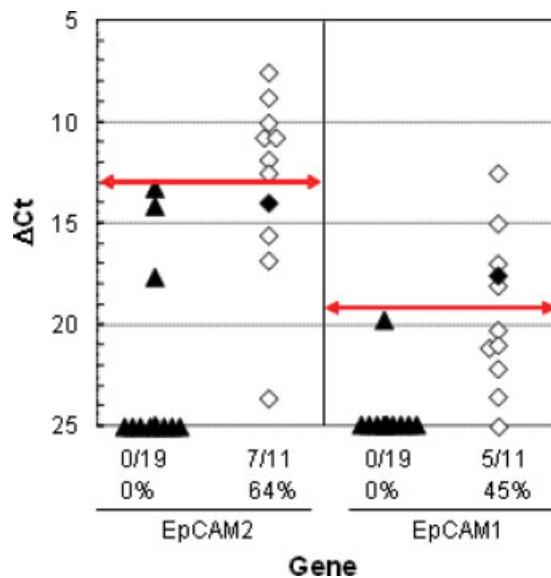


Figure 1. Expression levels for EpCAM2 (left panel), and EpCAM1 (right panel) measured by real-time RT-PCR. The denominators of fractions at the bottom of each panel refer to the number of subjects tested: 19 controls (full triangles), 11 prostate cancers (diamonds). The numerators refer to the number of subjects overexpressing EpCAM2 (left panel) or EpCAM1 (right panel) in controls (0 and 0), and prostate cancers (7 and 5), respectively. The full diamond refers to the only patient positive for EpCAM1 and negative for EpCAM2, barely below the threshold of significance. Note also that most control subjects show no detectable expression of EpCAM1 (16 of 19 subjects) or EpCAM2 (18 of 19 subjects). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

has the potential to increase sensitivity and specificity of CTC detection. We have previously utilized this system in combination with multi-marker real-time RT-PCR in a pilot study involving Stage IV breast cancer patients at the Medical University of South Carolina (MUSC). We detected breast cancer-associated overexpression in the peripheral blood of 13 out of 20 (65%) patients [5]. We also extended these observations to nonsmall cell lung cancer as 60% of patients with this tumor exhibited multimarker overexpression in their peripheral blood [6].

We enrolled 15 patients (11/15 of African-American ancestry) with advanced or metastatic AIPC in a clinical study sponsored by the General Clinical

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Causes and diagnosis of copper deficiency

To the Editor: I found the article by Huff et al. [1] on copper deficiency and myelodysplasia interesting and useful, but as a long time copper metabolism worker I found two problems in this article. First, copper deficiency was unexplained in three cases. The authors are aware of zinc ingestion as a cause of copper deficiency, and there is also the syndrome on which we have published [2] of unexplained high circulating zinc levels causing copper deficiency. Yet the authors do not mention questioning the patients about zinc intake, nor did they apparently measure serum or urine zinc. It is quite possible that one or more of the unexplained cases could be explained by a zinc mechanism.

The second problem has to do with the authors' lack of certainty on how to diagnose copper deficiency, for example their statement, "How to diagnose copper deficiency is not entirely clear." In their subsequent analysis of this area, they completely ignore a body of literature built up by our group on just that question. In our work, we induce mild copper deficiency with tetrathiomolybdate to achieve therapeutic objectives (reduction in angiogen-

TABLE I. Mutations in Albanian Hemophilia B Patients

Patient	Nucleotide position	Amino acid change	FIX:C (%)	Exons	Comments
HBA-1	Exon 1–6 deletion	–	<1	1–6	Inhibitor
HBA-2	Exon 1–6 deletion	–	<1	1–6	
HBA-3	119T > C IVS1 + 2	–	<1	INTRON 1	Splicing mutation
HBA-4	6491T > C IVS2 + 2	–	<1	INTRON 2	Splicing mutation, Novel
HBA-5	30153G > A	Gly234Ser	<1	7	
HBA-6	30153G > A	Gly234Ser	<1	7	
HBA-7	30953C > A	Pro278Thr	12	8	Novel

esis for cancer therapy, reduction in fibrosis, reduction in inflammation, treatment of autoimmune diseases) and have published on both clinical and animal model studies extensively (for reviews, see Refs. 3–6). We have established that the ceruloplasmin level is an excellent surrogate marker of copper status, and the various factors that can elevate ceruloplasmin levels, such as inflammatory disease, hormone therapy, etc., do not interfere with this use. The ceruloplasmin levels will come down in copper deficiency in all cases. Thus, using ceruloplasmin levels, rather than serum copper, is by far the best way to detect copper deficiency.

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First factor IX mutations in Albanian hemophilia B patients

To the Editor: Hemophilia B is an X-linked bleeding disorder, caused by deficient or defective factor IX (FIX) [1]. FIX is a vitamin K-dependent single-chain serine protease composed of 415 amino acids encoded by *F9*, which spans about 34 kilobases and contains 8 exons [1]. Among the 2,891 patients with hemophilia B listed in the 2004 updated database, 962 show unique molecular events (<http://www.kcl.ac.uk/ip/petergreen/haemBdatabase.html>). Furthermore, 91 gross deletions or insertions have been also identified.

Within the framework of the Twinning Program of the World Federation of Hemophilia, and working between the Hemophilia Centers of Vicenza (Italy) and Tirana (Albania), we assessed the genetic basis of hemophilia B in seven patients with hemophilia B from five families (Table I). Patients HBA-1 and 2 were brothers, while HBA-5 and 6 were cousins. Apart from HBA-7, all have severe disease (FIX < 1%).

Coding regions and intron/exon boundaries of the *F9* were PCR-amplified in nine reactions (methods available upon request) and the amplified fragments were sequenced on an ABI Prism 310 Genetic Analyser. All sequence changes were confirmed in both DNA strands and sought in 200 chromosomes.

A gross gene deletion spanning exons 1–6 was identified in the two brothers HBA-1 and HBA-2 and in their mother. This deletion occurred also in a Swedish [2] and an American patient, both without inhibitor, and in an Italian patient with inhibitor [3]. Thus, this type of deletion is likely to occur independently from a founder effect. Patient HBA-1 had allergic reactions upon treatment with FIX concentrates and at the time of sampling a modest inhibitor titer was present (0.9 BU), while no inhibitor was present in the brother. Two splicing defects (IVS1 + 2, nt. 119T > C and IVS2 + 2, nt. 6491T > C, novel) were also detected in association with a severe phenotype. In silico analysis by the splice site prediction program (<http://fruitfly.orth/cgi-bin/seq-tools/splice.pl>) of the novel IVS2 + 2 nt. 6491T > C predicts the loss of the original donor splice site, since the original probability score of 0.97 became nil after introducing the base exchange. The missense mutation Gly234Ser detected in patients HBA-5 and 6 was previously identified in an Italian patient with a severe phenotype [3]. Two additional patients, one French and one Italian [3], with Gly234Arg mutation and a severe phenotype have been reported, emphasizing the physiologic relevance of Gly234. A novel missense mutation (30953C > A; Pro278Thr) was identified in exon 8 in a patient with mild deficiency and also in his mother. In the database, only a different mutation in an UK patient with 30954C > A change (Pro278His) and a FIX level of 30% has been reported at the same codon. The Pro to Thr change occurs between an amino acid with a secondary amino group and a hydrophilic amino acid, with a hydroxyl group being introduced. Despite this apparently significant amino acid change, the mild laboratory and clinical phenotype suggests that although Pro278 is in the catalytic region, it is not drastically relevant to FIX activity. Both Gly234Ser and Pro278Thr changes occur at highly conserved amino acids, as demonstrated in murine, canine, and simian FIX (<http://www.ncbi.nih.gov/blast/>).

In conclusion, we have identified the first mutations (two novel) in Albanian hemophilia B patients. These results will help in establishing the Albanian hemophilia registry, along with the mutations identified in hemophilia A patients [4].

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