Differences between BG and DG and IFG respectively were significant (P <0.0001). Correlation between BG and DG ($r^2 = 0.79$) was not significantly improved with the ionic reference technique ($r^2 = 0.86$) ($P = 0.37$). Bland–Altman analysis comparing IFG to BG is shown in Fig. 1.

Microdialysis of adipose tissue adequately reflects BG in the setting of diabetes (4) and healthy volunteers. In this study in critically ill children, the correlation between BG and IFG was moderate. Bland–Altman analysis, however, indicated that the microdialysis technique is unsuitable to replace frequent blood sampling to safely monitor TGC in this patient group. The difference using IFG for BG was unacceptable in the clinically relevant glycemic ranges and this difference could be both negative or positive (Fig. 1); thus hyper- and/or hypoglycemia may be undiagnosed when only IFG is measured and therapeutic adjustments are based on this value. These results confirm those of previous studies (5) concluding that the correlation between BG and IFG of adult intensive care unit (ICU) patients is not as good as in healthy or diabetic individuals. It remains speculative whether this is a result of the particular patient population with disturbed microvasculature and treatment with vasoactive drugs. We used a flow rate of 1 μL/min to avoid the delay in detection of changes in IFG that occurs at lower flow rates and to avoid an insufficient hourly sample volume. Higher flow rates, however, can lead to local depletion of metabolites and dilution of the dialysate. Lower flow rates facilitate the capture of true interstitial glucose concentrations during glucose fluctuations. No drugs known to interfere with the used methodology of glucose measurements were administered.

Before microdialysis of the subcutaneous adipose tissue can be safely implemented for TGC in pediatric intensive care units, more studies are necessary to identify interfering factors and to optimize the performance of the current technology.

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Detection and Characterization of Putative Metastatic Precursor Cells in Cancer Patients

To the Editor:

Metastasis is the major cause of cancer-related death. Single disseminated tumor cells (DTC) can be detected in the bone marrow and peripheral blood years before the occurrence of clinically detectable metastases. Recent clinical studies, however, have clearly indicated that a significant fraction of breast cancer patients with DTC never develop distant metastases (1). Thus, DTC detected by sensitive immunocytochemical and molecular assays may be apoptotic or may lack stem cell properties and never give rise to an overt metastasis. The ability to detect and characterize viable DTC is therefore of utmost importance.

We applied a novel technique for the detection and ex vivo characterization of single viable DTC derived from epithelial tumors. Our technique for detection of specific secreted proteins, epithelial immunospot (EPISPOT), is an adaptation of the enzyme-linked immunospot assay. EPISPOT detects only viable tumor cells and can detect protein secretion at an individual cell level, allowing the direct determination of protein-secreting cell (SC) frequencies. Immunospots are the protein fingerprint left only by the viable SCs. Cell culture is needed for a sufficient amount of secreted marker proteins to accumulate to form immunospots; dying tumor cells do not secrete adequate amounts of protein and thus are not detected.

We first studied blood samples from breast and prostate cancer patients with gross metastases. SCs for the circulating tumor antigens mucin 1 (MUC1) or prostate-specific antigen (PSA) were detected in the majority of patients with metastatic breast (100%) and prostate (83.3%) cancer, respectively, whereas such SCs were not observed in healthy controls or in patients with benign prostatic hyperplasia (2). Consistent with our previous findings, the EPISPOT assay revealed viable DTC in the peripheral blood of 65% of prostate cancer patients, even in the absence of overt

metastases (stage M_0) (2), but the number of PSA-SCs in localized prostate cancer patients (median, 9; range, 2–197) was significantly lower ($P = 0.01$) than in patients with metastatic cancer (median, 29; range, 1–684), a finding that is in accordance with the different disease stages and total tumor burdens. Our findings, that MUC1- or PSA-SCs were present in patients with breast or prostate cancer but not in controls, suggest that these cells could be DTC released from the primary tumor.

To characterize DTC, we focused on fibroblast growth factor 2 (FGF2), a known stem cell growth factor also relevant for the in vitro growth of micrometastatic cells (3). We developed a dual fluorescent PSA/FGF2-EPISPOT to characterize PSA-SCs for the secretion of FGF2 and applied it to blood samples from 19 patients with localized prostate cancer. This study received ethics review board approval, and sample donors gave written informed consent. PSA-SCs were detected in 15 patients, and a subset of these SCs also secreted FGF2, suggesting that a significant fraction of DTC may secrete a factor potentially relevant to their outgrowth.

In addition, we tested whether the EPISPOT assay could also be applied to the analysis of bone marrow. In addition to MUC1, we screened for secretion of cytokeratin-19 (CK19), an intermediate filament of epithelial cells. Interestingly, our data provide the first evidence that CK19 can be secreted (Fig. 1A). The enumeration of both MUC1- and CK19-SCs allowed the detection of viable DTC in 90% and 54% of breast cancer patients with (Fig. 1B) and without (Fig. 1C) overt distant metastasis, respectively. These incidences are in the range of those obtained with sensitive reverse transcription PCR–based techniques (4). The number of DTC per sample was considerably lower in M_0-patients than M_1-patients, but most of the DTC detected in M_0 patients showed the $CK19^+/MUC1^-$ phenotype (Fig. 1C). DTC with this phenotype might have a particular biological potential, because recent findings of Gudjonsson et al. (5) suggested that $MUC1^-/CK19^+$ cells in the human breast may have stem cell–like properties. In addition to being structural proteins, cytokeratins play an unexpected role in influencing cell growth and size by regulating protein synthesis. A further characterization of the discovered $MUC1^-/CK19^+$ subset of DTC may be an important step toward the identification of the putative metastatic stem cells.

Taken together, our results demonstrate that DTC in patients with cancer of the prostate or breast are viable and heterogeneous with regard to the secretion of relevant proteins. Many secreted proteins influence metastatic progression (e.g., growth factors and proteases). The EPISPOT assay, a multiparameter technology that reveals a unique fingerprint of proteins secreted by single viable DTC, opens a new avenue in the understanding of the biology of the metastatic cascade. This information may be used in the future for improved molecular staging and treatment monitoring of cancer patients.

Fig. 1. (A), breast cancer cells. MUC1^{Alexa555}-immunospot (top), CK19^{Alexa488}-immunospot (middle), and MUC1/CK19^{yellow}-immunospot (bottom).

B

M1 Patients	CK19$^-$/MUC1$^+$	CK19$^+$/MUC1$^-$	CK19$^+$/MUC1$^+$
BC1	6	1	0
BC2	8	6	0
BC3	258	0	0
BC4	0	41	8
BC5	1	56	1
BC6	15	271	0
BC7	0	102	0
BC8	116	2	166
BC9	152	0	0
BC10	0	0	0
BC11	813	0	0
BC12	106	8	0
BC13	0	758	0
BC14	390	0	9
BC15	0	7	12
BC16	0	0	0
BC17	0	142	0
BC18	103	0	0
BC19	40	0	2
BC20	3	1	0
Total n DTC	2011	1395	198

C

M0 Patients	CK19$^-$/MUC1$^+$	CK19$^+$/MUC1$^-$	CK19$^+$/MUC1$^+$
BC21	8	254	0
BC22	2	0	1
BC23	7	92	0
BC24	0	0	0
BC25	0	0	0
BC26	17	159	0
BC27	8	0	0
BC28	0	0	0
BC29	0	0	0
BC30	0	0	0
BC31	3	3	0
BC32	0	1	0
BC33	0	0	0
BC34	0	0	0
BC35	3	0	0
BC36	0	0	0
BC37	7	0	0
BC38	0	0	0
BC39	1	2	0
BC40	0	0	0
BC41	32	1	0
BC42	0	0	0
BC43	1	28	0
BC44	1	1	1
BC45	0	11	0
BC46	0	23	0
BC47	0	6	0
BC48	0	1	0
BC49	0	0	0
BC50	0	7	0
BC51	0	0	0
BC52	0	0	0
BC53	0	0	0
BC54	0	6	0
BC55	0	0	0
BC56	0	2	0
BC57	0	0	0
Total n DTC	90	597	2

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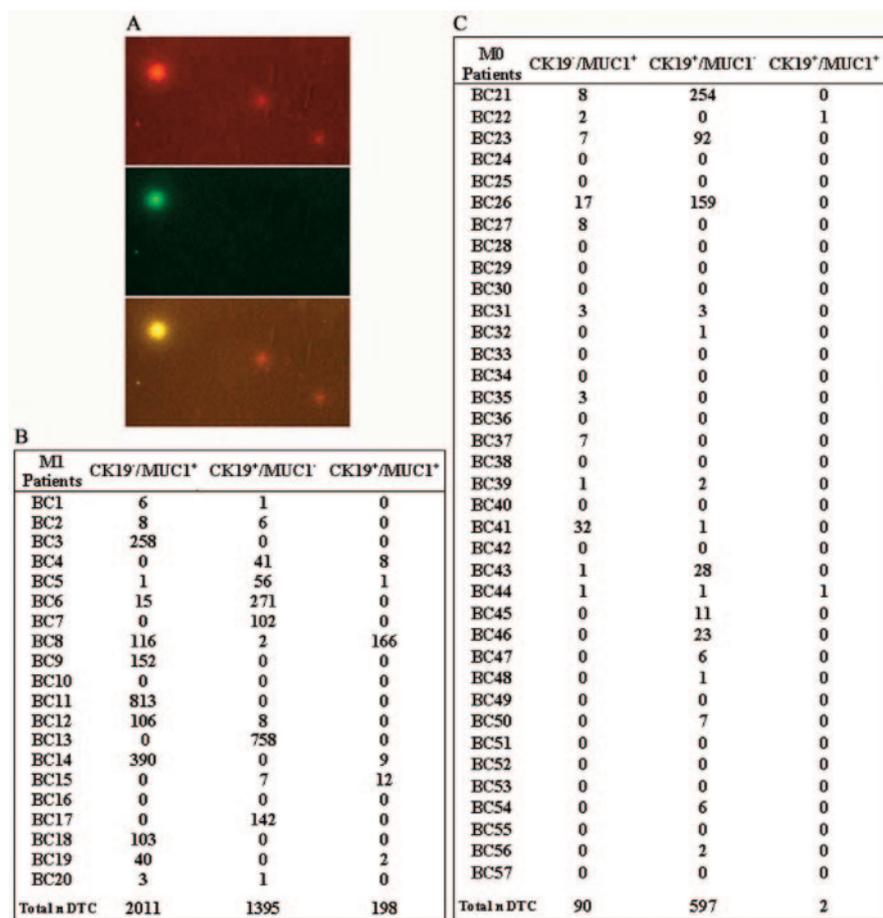
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The −50G>T Polymorphism in the Promoter of the *CYP2J2* Gene in Coronary Heart Disease: The Ludwigshafen Risk and Cardiovascular Health Study

To the Editor:

Cytochrome P450 epoxygenases metabolize arachidonic acid to epoxyeicosatrienoic acids (EETs), which possess various biological properties with a role in coronary vascular function. Therefore, the cytochrome P450, family 2, subfamily J, polypeptide 2 (*CYP2J2*) gene, which encodes CYP2J2, the predominant epoxygenase isoform involved in EET formation in the human heart, has been considered a candidate gene for coronary artery disease (CAD) *(1)*. A frequent G-to-T polymorphism at position −50 leads to a 50% reduction in *CYP2J2* promoter activity *(2, 3)*. In a cohort of 544 patients, this polymorphism was independently associated with an increased risk of CAD *(3)*.

We investigated whether the −50G>T polymorphism is associated with angiographic CAD, myocardial infarction (MI), or mortality in the Ludwigshafen Risk and Cardiovascular Health (LURIC) cohort, which included white patients hospitalized for coronary angiography between June 1997 and May 2001. The study was approved by the ethics review committee at the Landesärztekammer Rheinland-Pfalz (Mainz, Germany). A detailed description of LURIC has been published *(4)*. In total, the LURIC cohort includes 3243 individuals, 2547 (78.5%) with angiographically proven CAD ("CAD patients") and 696 (21.5%) individuals without CAD ("CAD controls"). Information on vital status was obtained from local registries. Another control group ("blood donor controls") included a cohort of 960 healthy blood donors [male: female 1:1; mean (SD) age 58 (5) years] with an ethnic and geographic origin comparable to that of the LURIC cohort.

Genomic DNA was prepared from EDTA-anticoagulated peripheral blood by salting-out or a commercial system (DNA Blood Mini Kit; Qiagen). The −50G>T polymorphism (rs890293) was genotyped by PCR and *Alu*I restriction fragment-length analysis *(3)*. Associations between categorical variables were examined by χ^2 testing or logistic regression analysis, and covariable adjustments were done for age, sex, type 2 diabetes, body mass index, smoking, hypertension, and dyslipidemia. We used the Cox proportional hazards model to calculate hazard ratios and 95% confidence intervals. The criterion for statistical significance was $P < 0.05$. The SPSS statistical package (SPSS Inc. Ver. 11.5) was used.

Genotypes fulfilled Hardy-Weinberg equilibrium in each cohort. There were no significant differences in the frequencies of genotypes for CAD patients, CAD controls, and blood donor controls (Table 1). None of the logistic regression models revealed a significant association between the *CYP2J2* polymorphism and CAD, regardless of whether or not we adjusted for conventional cardiovascular risk factors (data not shown). Very similar results were obtained when we compared the *CYP2J2* genotypes in patients with previous MI vs CAD patients without MI (Table 1). Results were also not significant in any respect when we screened for relationships between *CYP2J2* and early manifestations of CAD or MI. Among the 3243 LURIC study participants included in this examination, 496 deaths (15.4%) occurred during a median observation time of 5.45 years. The analysis for cardiovascular mortality included a total of 3229 individuals, of whom 333 (10%) died from cardiovascular causes. No deaths occurred in individuals with the TT genotype. Therefore, Cox models comparing only the GG with the GT genotypes were calculated. Regardless of whether or not we adjusted for age, sex, and conventional risk factors, the *CYP2J2* polymorphism showed no association with mortality from all causes or with cardiovascular mortality (data not shown). Because EETs have been reported to inhibit vascular inflammation, we analyzed the relationship between systemic markers of inflammation such as sen-