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Circulating tumour cells as a predictive factor for response to systemic chemotherapy in patients with advanced colorectal cancer

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ABSTRACT

Circulating tumour cells (CTC) can be traced in patients with different types of cancer. The aim of this study was to detect CTC in patients with advanced colorectal cancer and whether CTC are still detectable after systemic chemotherapy. Blood from 34 patients with advanced colorectal cancer was analysed for the presence of CTC before chemotherapy was given and after 3 months. Eleven patients demonstrated a tumour remission after chemotherapy. In 6 cases CTC were detectable before but not after initiation of chemotherapy. Ten patients demonstrated a progression. In 5 cases CTC were detected before and after chemotherapy. Our data suggest that the detection of CTC will help to identify patients responding to chemotherapy or with a risk of a therapy failure.

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1. Introduction

The presence of circulating tumour cells (CTC) in the peripheral blood of patients with colorectal cancer (CRC) has already been noted (Cohen et al., 2006; Molnar et al., 2003; Zieglschmid et al., 2007) and their clinical relevance has been described extensively as an independent prognostic marker for disease-free survival (Guller et al., 2002; Koch et al., 2006; Thorban et al., 2006). CTC could be used as surrogate markers to monitor drug effects and clinical status (Elshimali and Grody, 2006). If a patient responds to an administered therapy, CTC should be no longer detectable in peripheral blood. Therefore, patients with CTC might be potential candidates for relapse and therapy failure.

Besides chemotherapeutic agents like 5-fluorouracil (5-FU), folinic acid (FA), oxaliplatin and irinotecan, targeted therapies including therapeutic antibodies are administered in combination or in chemotherapeutic resistant patients. Bevacizumab, panitumumab and cetuximab are already approved and the latter is used in daily practice with metastatic CRC patients as a target antibody for epidermal growth factor receptor (EGFR) (Pfeiffer et al., 2007). EGFR, a transmembrane receptor tyrosine kinase is activated by binding of the natural ligand epidermal growth factor (EGF) or transforming growth factor α (TGFα). The therapeutic antibodies cetuximab and panitumumab bind to EGFR with high affinity and therefore prevent the binding of EGF or TGFα. This results in reduced

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receptor tyrosine kinase activity and in reduced cell proliferation, cell survival and cell invasion.

In most studies patients with metastasised CRC have an extended time to tumour progression if they were treated with antibody based therapies independent of the EGFR status of the primary tumour (Meropol, 2005; Pfeiffer et al., 2007; Zhang et al., 2006). EGFR expression analysis by immunohistochemical staining of tumour sections correlates only in some cases with tumour response to EGFR targeted therapy and is therefore of limited help for the identification of patients possibly responding to EGFR antibody therapy (Chung et al., 2005; Cunningham et al., 2004; Nygren et al., 2005; Vallböhmer et al., 2005). Genetic alterations of the extracellular domain of the EGF receptor or different EGFR expression pattern in the primary tumour and metastasis or CTC can both lead to treatment failure despite immunohistochemically detected EGFR expression in tumour biopsies (Bralet et al., 2005; Italiano et al., 2005; Scartozzi et al., 2004).

This pilot study was performed to determine the predictive value of CTC. Blood obtained from CRC patients was examined by immunomagnetic enrichment with subsequent RT-PCR techniques for the circulation of tumour cells before and after therapy. Peripheral blood from patients with chronic inflammatory bowel disease was used as a control group to demonstrate the specificity of CTC detection on patients with colorectal cancer. We hypothesized that successful chemotherapy should lead to a decreased number of CTC, which will then lead to a negative PCR result. PCR results were compared with clinical responses. Another aim of the study was to determine the expression of EGFR variants on CTC to identify additional patients who might not profit from EGFR antibody targeted therapies because of the deletion of extracellular binding sites.

2. Patients and methods

2.1. *Patients and study design*

A total number of 34 CRC patients (20 males, 14 females) with advanced disease were enrolled before and 3 months after chemotherapeutic treatment at the Department of Gastroenterology, Hepatology and Endocrinology of the Hannover Medical School, Germany. Peripheral blood from 64 patients with Crohn's disease (13 males, 19 females) or ulcerative colitis (19 males, 13 females) was also tested to evaluate the specificity of the test used. Informed consent was obtained from all patients prior to obtaining blood samples; the Ethics Committee of the Hannover Medical School approved the study protocol. The following parameters were recorded for all patients: age, sex, diagnosis, serum carcinoembryonic antigen (CEA) and the presence of CTC. Additionally the following parameters were recorded for the CRC patients: TNM classification of the primary tumour, status of disease clinically validated by computer tomography before and 3 months after treatment classified according to WHO criteria.

2.2. *Tumour cell enrichment and multiplex RT-PCR*

Peripheral blood (2 × 5 ml) from each patient was collected in EDTA tubes (Sarstedt AG & Co, Nümbrecht, Germany) and processed within 4 h for the enrichment of CTC and subsequent expression analyses. For the detection of CTC the AdnaTest ColonCancerSelect and AdnaTest ColonCancerDetect (AdnaGen AG, Langenhagen, Germany) were employed according to the manufacturer's protocol. The combination of immunomagnetic tumour cell enrichment and the analysis of tumour-associated transcripts EGFR, CEA and GA733-2 (gastrointestinal tumour-associated antigen 733-2) by multiplex RT-PCR were previously described (Zieglschmid et al., 2005). Actin was amplified as an internal PCR control. PCR products were analysed with DNA 1000 assays using an Agilent 2100 Bioanalyzer (Analysis Software 2100 expert, version B.02.03.SI307, Agilent Technologies, Böblingen, Germany). A threshold of <0.1 ng/μl was defined as negative. The cDNA was also used for the detection of different extracellular EGFR variants.

2.3. *PCR amplification of extracellular EGFR variants from CTC*

The PCR for the detection of extracellular EGFR variants in CTC was previously described (Lankiewicz et al., in press). In brief, the PCR was performed under the following conditions: Two primer pairs (EGFR P1 5′-AAACTGCACCTCCATCAGTG-3′/EGFR P2 5′-ATTCGTTGGACAGCCTTCAAG-3′ and EGFR P3 5′-GTCCAGTATTGATCGGGAGAGC-3′/EGFR P4 5′-GAGCCGTGA TCTGTCACCAC-3′) were designed to span exon 9 to exon 16 and exon 1 to exon 8, respectively; 1.0 μM of each primer and 25 μl of HotStarTaq Mix (Qiagen GmbH, Hilden, Germany) were used. The PCR program was set for 15 min at 95 °C, followed by 45 cycles of 94 °C for 30 s, 60 °C (for P1/P2) or 63 °C (for P3/P4) for 30 s and 72 °C for 1 min (for P1/P2) or 2 min (for P3/P4), followed by a final step of 72 °C for 5 min. The analyses of the PCR fragments were performed with DNA 1000 (for fragments detected with P1/P2) or DNA 7500 assays (for fragments detected with P3/P4) and an Agilent 2100 Bioanalyzer (Analysis Software 2100 expert, version B.02.03.SI307, Agilent Technologies, Böblingen, Germany) according to the manufacturer's instructions.

2.4. *Determination of serum CEA*

CEA levels were determined using standard assays (Modular Analytics <E 170>, Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The results were evaluated using a threshold of >3 mg/l defined as positive.

3. Results and discussion

3.1. *Detection of CTC and determination of CEA serum levels in CRC patients and patients with inflammatory bowel diseases*

Two blood samples were taken from patients with advanced colorectal cancer, one before the start of chemotherapy and one after 3 months. CTC were analysed in both samples and CEA levels were determined. CTC were detected in 20/34 (59%) patients prior to chemotherapy (Table 1); 28/34 patients

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