RESEARCH REVIEW

Quantitative RT-PCR Detection of Colorectal Tumor Cells in Peripheral Blood—A Systematic Review

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Background. Twenty percent to 40% of patients with node-negative colorectal cancer die of metastatic disease. Detection of cancer cell dissemination has been proposed as a tool to select patients at highest risk for recurrence. In this review, we summarize the evidence for detection with quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assays of circulating tumor cells (CTCs) in peripheral blood of colorectal cancer patients.

Materials and methods. Pubmed and Embase were systematically searched for all English publications relevant to circulating cancer cells, peripheral blood, quantitative RT-PCR (q-RT-PCR), and colorectal cancer. Cross-references and the “related articles” function were used to broaden the search. Manuscripts reporting on the results of nonquantitative RT-PCR assays were excluded. The study methodology, CTCs detection rates in peripheral blood, and prognostic value were reviewed.

Results. Twelve manuscripts on qRT-PCR were retrieved. Stage dependence was found for detection of CTCs in four of 10 studies. From univariate analysis performed for disease-free survival and overall survival in 4 of 12 studies, there was evidence (P < 0.05) for an effect of the detection of CTCs with qRT-PCR. None of the included trials identified detection of CTCs in peripheral blood as an independent predictor of survival.

Conclusion. Quantification of CTCs in peripheral blood holds promise in predicting stage and outcome in colorectal cancer patients. At present, evidence from the literature is too scarce to integrate quantitative RT-PCR assays to detect CTCs into the management of colorectal cancer.

Key Words: neoplasm circulating cells [C23.550.727.650.645]; real-time RT-PCR; quantitative RT-PCR; reverse transcription polymerase chain reaction [E05.393.620.500.725]; colorectal neoplasms [C04.588.274.476.411.307].

INTRODUCTION

Metastatic lesions and not the primary tumors are the leading cause of death in patients with carcinomas [1]. After curative surgery, 20% to 40% of patients with so-called localized colorectal cancer die of metastasis [2, 3]. During this metastatic process, cancerous cells detach from the primary tumor and migrate to secondary organs. Over almost a century, circulating tumor cells (CTCs) have been a focus of research in the measurement of this phenomenon.

Initially, detection of CTC in blood of cancer patients was achieved by cytology, immunocytochemistry (ICC), and flow cytometry based techniques. Cytology allowed detection of CTCs in the blood in 25% to 67% of examined patients [4–8]. The combination of cytology and ICC permitted to detect CTCs in four of 42 patients undergoing resection for colorectal cancer [9]. These three techniques have high specificity but limited sensitivity [10].

Reverse transcriptase-polymerase chain reaction (RT-PCR), a modification of classic PCR, was designed to detect mRNA. RT-PCR identification of mRNA coding for CEA, CK20, k-ras mutations, CD44 splice variants, and other proteins is capable of identifying cultured colorectal carcinoma cells in normal blood with a sensitivity of 1 in 10⁷ leukocytes (1–10 mL of blood) [11–16]. This represents a level of sensitivity three orders of magnitude greater than ICC [17]. Multimarker RT-PCR has further enhanced the assay sensitivity. The use of more than one marker gene eliminates some of the problems associated with single-marker detection techniques, such as tumor cell heterogeneity,
clonal selection during tumor metastasis, and variable expression of individual genes within tumor cells [18]. This enhanced sensitivity, however, is at the cost of lowering specificity if detection of just one of these markers is considered a positive result.

The detection of CTCs in perioperative venous blood with nonquantitative RT-PCR assays was found to have prognostic significance in some studies [19–26]. However, the majority of studies failed to show a predictive value. Inconsistent findings can be explained by a lack of statistical power and inadequate length of follow-up. Other explanations for inconclusive results are high background expression of the selected markers resulting in false-positive results and the genetic heterogeneity of tumor cells. Moreover, traditional RT-PCR assays are at most a semiquantitative measurement of cancer cell load in the peripheral blood. Quantitative RT-PCR (qRT-PCR) is another modification of the PCR-principle and eliminates at least some of the problems associated with traditional RT-PCR. These assays, whether TaqMan or SyBGreen based platforms, allow for quantification of cancer cell load in peripheral blood and determination of cutoff values indicating mRNA expression levels of clinical relevance in cancer patients compared with healthy subjects [27]. Using fluorescent dyes that preferentially bind to double-stranded DNA, quantification of mRNA expression of a specific gene in a sample can be accomplished when it is compared with a control sample [28].

An additional advantage of qRT-PCR is the possibility to take into account variations in RNA and/or cDNA quality by quantifying housekeeping genes and subsequent normalization of marker concentration to that of the housekeeping gene [29].

In this manuscript we review the present evidence for quantification of CTCs in peripheral blood of colorectal cancer patients with qRT-PCR.

### MATERIALS AND METHODS

Pubmed and Embase were systematically searched for English articles published between January 1993 and January 2008. The following search terms were used for Pubmed: "neoplasm circulating cells" [MeSH Terms] AND "colorectal neoplasm" [MeSH Terms] AND "peripheral blood" [All Fields] AND "reverse transcriptase-polymerase chain reaction" [MeSH Terms] OR "quantitative pcr" [All Fields] OR "real-time pcr" [All Fields]) NOT "leukemia" [All Fields]. Search terms for Embase were: (("real time polymerase chain reaction"/syn OR "real time polymerase chain reaction") OR ("reverse transcriptase polymerase chain reaction") AND ("colorectal cancer"/syn OR "colorectal cancer") OR "colon cancer"/syn OR "colon cancer") OR ("rectum cancer"/syn OR "rectum cancer") AND ("tumor embolism"/syn OR "tumor embolism") AND [english]/lim AND [1993-2008]/py. We reviewed abstracts of all original articles to select those studies that used a quantitative PCR assay to detect colorectal cancer cells in peripheral blood. To avoid potential double counting of patients, the most recently published paper of any research group that maximized the overall sample number was retained. Manuscripts reporting on nonquantitative RT-PCR assays and reviews were excluded. Of eight manuscripts, the full text was acquired. We were able to identify another four studies by using the "related articles" function and looking for cross-references in these studies. Primary outcomes of interest were study methodology and prognostic impact on disease-free and overall survival. Relevant data of the 12 included studies were extracted using a standard fill-out form. A meta-analysis was not performed because inter-study methods, study population, and results differed greatly [30, 31].

### RESULTS

#### Clinicopathological Characteristics

One of 12 original articles in this review was a multicenter study (Table 1). All studies included adenocarcinomas of the colon and rectum.

#### Timing and Origin of Blood Sample

In 8 studies, peripheral blood samples were taken prior to treatment (Table 2). In 11 studies, venous blood samples were used to quantify CTCs. A single

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<th>Year</th>
<th>Multicenter?</th>
<th>n</th>
<th>Tumor type</th>
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<tr>
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<td>1999</td>
<td>No</td>
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<tr>
<td>Guller et al.</td>
<td>2002</td>
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<td>39</td>
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<td>TNM stage I–IV</td>
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</table>

CR = colorectal; NR = not reported.
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<th>Probe</th>
<th>n PCR cycles</th>
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<th>Dichot. cut-off</th>
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<th>Timing of samples</th>
<th>Origin of blood</th>
<th>Separation technique</th>
<th>Magnetic cell separation</th>
<th>Detection</th>
<th>Po/Fu increased detection?</th>
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</table>

NR = not reported; CK = cytokeratin; CEA = carcinoembryonic antigen; ProtM = protease M; GCC = guanyl cyclase C; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; PBGD = porphobilinogen deaminase; β-2-M = β-2-microglobulin; RCL = red cell lysis; DC = density centrifugation; Pre = Preoperative blood; I = intraoperative blood; Po = postoperative blood; Fu = at follow-up; NBC = nucleated blood cells; PVB = peripheral venous blood; MVB = mesenteric venous blood.
study used blood from the radial artery [32]. Intraoperative mesenteric venous blood samples were taken in two studies. In three studies, the timing of blood samples was not reported. Volume of blood samples varied from 3 to 20 mL (median: 10 mL).

**Marker Genes**

CEA, CK20, and guanylyl cyclase C were used as marker genes for PCR in 7, 9, and 2 of 12 studies respectively (Table 2). In 5 studies, a multimarker assay was used for cancer cell detection. Four different control genes were used for normalization: glyceraldehyde 3-phosphate dehydrogenase [32–35], β-actin [36, 37], porphobilinogen deaminase [29, 38, 39], and β2-microglobulin [40]. In two studies, normalization procedure and control genes were not reported [41, 42]. When identical marker and control genes were chosen for amplification, primers and probe sequence combinations differed between studies.

**Cell Separation Technique**

Prior to RNA extraction, the mononuclear fraction was enriched with density gradient centrifugation in seven studies [34, 36–42] (Table 2). In two studies, additional efforts were made to further improve test assay sensitivity using a combination of immunomagnetic enrichment strategies [38, 40]. Following density centrifugation, Guo et al. [40] equally divided each sample of peripheral blood mononuclear cells into 3 parts and used CD45 immunomagnetic beads and/or Ber-EP4 immunomagnetic beads to enrich for colon cancer cells. Dandachi et al. [38], on the other hand, used a anti-HEA125 magnetic microbead conjugate to positively enrich for epithelial cells after red cell lysis.

**Cutoff Strategies**

In all studies, different cutoff strategies were used for discrimination between presence or absence of CTCs in blood samples of colorectal cancer patients (Table 2). For all studies, a maximum number of PCR amplification cycles was determined at the start of the assay. If after a predefined number of cycles no fluorescent signal was detected on amplification plots, the marker mRNA was assumed absent in the blood samples. The median threshold cycle (Ct) for the included studies was 45.

In 7 studies, additional statistical criteria were explored to dichotomize between a positive and a negative blood sample. Among these *a posteriori* cutoff strategies used were a Ct lower than 40 [36, 39] and the 95% confidence interval [35] or the 99% percentile of log-normal distribution [29] of normalized copy numbers in healthy/benign donors.

**Stage-Dependent Detection of Circulating Tumor Cells**

CTCs were detected preoperatively in 0.8% to 100% of patients with colorectal adenocarcinoma (Table 2). Three out of 10 studies found a stage-dependent detection of CTCs in peripheral blood [35, 37, 40] (Table 3). In the study by Miura et al. [37], patients with Dukes D disease had significantly higher transcript levels in their peripheral blood *versus* controls on univariate analysis (*P* < 0.0001). In the only multicenter trial CEA and CK20 mRNA positivity rates in both peripher-

### TABLE 3

<table>
<thead>
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<th>Follow-up (mo)</th>
<th>DFS (P value)</th>
<th>OS (P value)</th>
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<td>2002</td>
<td>No</td>
<td>31 (median)</td>
<td>0.001 (CR, mv)</td>
<td>0.0035 (CR,uv)</td>
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<td>0.03 (BGW, uv)</td>
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<td>Yes</td>
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<td>2004</td>
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<td>0.001 (W,uv*)</td>
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<tr>
<td>Chen et al.</td>
<td>2006</td>
<td>No</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guo et al.</td>
<td>2007</td>
<td>Yes</td>
<td>0.003 (χ²)</td>
<td>30 (median)</td>
<td>0.021 (LR,uv)</td>
</tr>
<tr>
<td>Dandachi et al.</td>
<td>2008</td>
<td>No</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giribaldi et al.</td>
<td>2009</td>
<td>No</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xu et al.</td>
<td>2010</td>
<td>No</td>
<td>&gt;0.05 (χ²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iinuma et al.</td>
<td>2011</td>
<td>Yes</td>
<td>0.99 (χ²)</td>
<td>40 (median)</td>
<td>0.08 (LR, uv)</td>
</tr>
<tr>
<td>Friederichs et al.</td>
<td>2012</td>
<td>—</td>
<td>—</td>
<td></td>
<td>0.06 (CR, uv)</td>
</tr>
</tbody>
</table>

NR = not reported; mn = mean; md = median; χ² = chi-square test; F = Fisher’s PLSD test; K-W = Kruskal-Wallis one-way analysis; mv = multivariate analysis; uv = univariate analysis; CR = Cox proportional hazard regression analysis; BGW = Breslow-Gehan-Wilcoxon test; W = Wilcoxon signed rank test; LR = Log Rank test.

* Postoperative day 14.
eral and tumor drainage blood were stage-dependent on univariate analysis ($P = 0.003$ and $P < 0.001$, respectively) [35]. An increased postoperative detection was found in three of four studies that analyzed postoperative peripheral blood samples [29, 32, 34].

**Prognostic Value (Table 3)**

Five studies analyzed the prognostic impact of CTC detection in peripheral blood (Table 3). In the earliest by Guller et al. [36], the detection of tumor cells in peripheral blood and/or mesenteric blood and/or peritoneal lavage fluid had a significant adverse impact on disease-free ($P = 0.001$, multivariate) and overall survival ($P = 0.0035$, univariate). In the same study, disseminated colorectal cancer cells were found in the peritoneal lavage fluid of seven patients, but not in blood specimens. Five of these seven patients experienced recurrence of their disease. In the study by Ito et al. [32], univariate analysis of Kaplan-Meier survival curves demonstrated disease-free survival of patients with positive CEA mRNA in postoperative blood to be significantly shorter than in cases negative for CEA mRNA ($P = 0.03$). Chen et al. [34] showed that detection of more than 100 free tumor cells (FTC) per $10^6$ nuclear blood cells 2 weeks after surgery was associated with worse disease-free survival ($P = 0.001$, univariate) and tumor-related mortality ($P = 0.039$, univariate) (Table 3). In the study by Inuma et al. [35], Log rank testing of Kaplan-Meier curves showed a significantly poorer prognosis for patients with a positive qRT-PCR in peripheral blood for disease-free and overall survival ($P = 0.021$ and $P < 0.0001$, respectively). Univariate Cox proportional hazard regression analysis did not reveal a significant impact on disease-free and overall survival ($P = 0.075$ and $P = 0.078$, respectively). The most recent study by Friederichs et al. [39] updates previously published data [43] after a median follow-up of 40 months. The trend toward worse overall survival in the CK20-positive patients is not statistically significant on univariate analysis ($P = 0.08$).

None of the included trials identified detection of CTCs in peripheral blood as an independent predictor of overall survival.

**DISCUSSION**

**Detection of Circulating Tumor Cells**

In recent years, many studies have addressed the feasibility and clinical impact of detecting minimal residual disease in lymph nodes, bone marrow, peritoneal cavity, liver, and blood of patients with colorectal cancer. The peripheral blood in particular has practical advantages over other compartments since it can be reached by a routine venipuncture. Although conflicting results have been obtained, several studies suggest the potential of CTC detection in the peripheral blood for cancer prognosis and follow-up.

Different techniques have been used to detect, isolate, and characterize CTCs and altogether they can be divided into direct and indirect methods. Direct methods aim at providing a direct identification of malignant features of CTCs through cytopathological or genome analysis (e.g., fluorescence *in situ* hybridization, CGH, mutation analysis) [44]. Since CTCs are present at very low concentrations in the peripheral blood, direct methods necessitate additional enrichment of CTC with density-gradient centrifugation (e.g., Ficoll, Lymphoprep [Axis-Shield PoC, Oslo, Norway], Onco-Quick [Greiner Bio-one, Kremsmünster, Austria]) or membrane filtration (e.g., ISET). These enrichment procedures are independent of surface antigens and tend to conserve cell morphology allowing cytopathological examination.

Indirect methods on the other hand target epithelial or organ-specific markers, which identify cells from epithelial origin in the blood but do not provide proof of the cancerous nature of these cells. Indirect detection of circulating epithelial cells can be obtained with immunomedi ated, e.g., MACS, macro-iron beads, magnetic beads, and ferrofluid-based systems (Cellsearch), and molecular methods (e.g., PCR, RT-PCR, and qRT-PCR) [44]. In this systematic review, the present evidence for quantification of CTCs in peripheral blood of colorectal cancer patients with qRT-PCR is summarized.

**Markers**

For RT-PCR studies, an appropriate marker gene has to meet at least two requirements. First, the marker has to lack expression in tissues in which the disseminated tumor cells need to be detected [45]. Therefore, most marker genes chosen to detect cancer cells are involved in epithelial differentiation or malignant transformation [46]. Second, the expression of the marker in tumor or originating tissue must be relatively high. A common problem for indirect detection of CTCs in peripheral blood is that no available markers are 100% tumor- or tissue-specific [46]. In the past, many marker genes such as CEA [47], mucin 1, gastrointestinal tumor-associated antigen 733.2 (GA 733.2), CD44 splice variants, human telomerase reverse transcriptase [47], cytokeratin 8, CK18, CK19 [47], and CK20 [47] have been used to detect minimal residual disease in colorectal cancer. Of these, CEA, and CK20 are by far the most common molecular markers used to study CTCs. In the retrieved qRT-PCR studies protease M, guanylyl cyclase, CEA, CK19, and CK20 were used as marker genes to detect CTCs in the peripheral blood of colorectal cancer patients.

Protease M (Prot M, Zyme, Neurosin) codes for a trypsin-like serine protease isolated from colon and ovarian cancer cell lines [48, 49]. This marker was only
used in the study by Schuster et al. [29]. Despite high expression in all seven colon cancer cell lines tested and low background expression in blood-spiking experiments with COLO 205 cells, they found that the expression of protease M in circulating colorectal cancer cells was insufficient.

Guanylyl cyclase C, the receptor for *E. Coli* heat-stable enterotoxin, is expressed exclusively in normal intestinal mucosal cells, adenomatous polyps, and colorectal carcinomas but not in extraintestinal tissues or tumors [50]. It has been proposed as a selective marker for metastatic colorectal tumors in extraintestinal tissues, including peripheral blood [51, 52], and was used in two of the retrieved studies [33, 34].

High expression of intermediate filament CK19 in primary Dukes B colorectal adenocarcinoma is associated with a significant reduction of disease-free and overall survival [53]. The expression of CK20 is entirely confined to the gastrointestinal epithelium, urothelium, and Merkel cells [54]. The expression of CK20 has not been found in normal hematopoietic cells [55]. Nonetheless, use of CK19, CK20, and other cytokeratins as a marker in the assessment of CTCs in peripheral blood has been questioned on the basis of high background expression in healthy volunteers [13, 26, 56, 57]. Careful selection of primer-probe combinations plays an essential role in minimizing the number of false positives in control samples. Furthermore, the ideal enrichment strategy (e.g., RBC lysis, density centrifugation, magnetic bead separation) and ultimately RNA extraction technique is still under debate, as undoubtedly both influence background expression.

Similarly, for CEA, false positive results were recorded in up to 33% of control samples [25]. Multimarker qRT-PCR assays deal at least partially with tumor cell heterogeneity and false positive results by determination of a cutoff value. Historically, a PCR result is considered positive when a predefined Ct is reached for a marker gene. The determination of the Ct is usually orientated at the maximum background level in a healthy/benign control population. However, sometimes the determination of a reliable cutoff value to differentiate mRNA from rare CTCs and that from illegitimate transcription in peripheral blood leukocytes is virtually impossible [29].

**Do Preoperative or Postoperative CTC Levels Predict Relapse?**

The majority of series published on detection of CTC have studied preoperative or intraoperative blood samples. Many of these studies failed to find an independent prognostic value.

Inconsistencies in study results could be explained by the fact that preoperative CTCs rather reflect intermittent shedding of tumor cells than true metastatic potential. Metastasis is an inefficient process where circulating malignant cells are eliminated almost immediately from the blood. Moreover, the majority of the cells that survive, arrest, and extravasate in lymph nodes, lungs, liver, and bone marrow will remain dormant for many years and never cause relapse of cancer.

Two recently published RT-PCR studies found postoperative detection of CTCs to be an independent predictor of disease-free (DFS) and disease-specific survival. Both studies used a nonquantitative assay to detect either CEA and CK20 [20] or CK20 only [21] mRNA transcripts in the postoperative blood of patients with node-negative colorectal cancer. In this review, only Chen et al. [34] found a worse DFS and overall survival for postoperative detection of CTCs, although two other studies also found an increased postoperative detection of CTCs [29, 32, 34]. This discrepancy can be explained by the fact that intraoperative manipulation of the tumor and colon not only results in an increased shedding of cancerous cells, but also introduces normal epithelial cells in the peripheral blood participating in an increased postoperative detection depending on the markers used [58].

In nonmetastatic breast cancer, all patients relapsed when a more than 10-fold increase of CTCs was detected with MAINTRAC (Laser Scanning Microfluorimetry) after start of chemotherapy [59]. This finding points that the variations in circulating cancer cell counts during or after treatment might be a better predictor of outcome than pretreatment levels. Therefore, it is sensible that further studies focus on the quantification of CTCs in peripheral blood at various time intervals after curative surgery. Detection of CTC in patients without clinically detectable disease could prove to be a powerful surrogate marker for tumor activity or relapse. As a consequence, it could become an important tool in postoperative patient selection and monitoring of adjuvant therapy.

**Interpretation of Quantitative RT-PCR Studies**

In the study by Inunuma et al. [35], detection of CTCs in tumor-draining blood and not peripheral blood (Table 3) was found to be an independent predictor of disease-free and overall survival ($P = 0.031$ and $P = 0.042$, respectively) [35]. A plausible explanation for this result could be the lower qPCR positivity rate in peripheral blood versus tumor-draining blood for overall survival (17/167 versus 57/167) and for disease-free survival (8/128 versus 32/128) resulting in insufficient events in proportion to the sample size. Another explanation could be that detection of CTCs in the peripheral blood is more likely to be influenced by a first-pass effect of the liver [60]. Nevertheless, Kaplan-Meier survival curves of overall and disease-free survival were significantly shorter in patients with CEA/CK20 mRNA positive peripheral or tumor-draining blood than in cases negative for these markers. None of the other
included studies found a significant predictive value of the detection of CTCs in peripheral blood.

However, we hope that updates of the included studies will appear in the near future since re-analysis of survival after a longer median follow-up could reveal a trend toward worse overall survival in the marker positive patients. Interpretation and comparison of reviewed trials is particularly challenging because published data have a high degree of variability in terms of timing and number of blood samples, methodological and technical features of circulating tumor cell detection (density-gradient centrifugation or RBC lysis, with or without (magnetic) cell enrichment, different PCR platform, primers and probes, and type and number of tumor markers analyzed [61]. Standardization of protocols and the conducting of large multicentric trials are urgently needed.

**Tumor-Cell Heterogeneity**

Another explanation for inconclusive findings from CTC trials lies in the heterogeneity of gene expression in cancer cells in both primary tumor and peripheral blood. Detection of CTCs in peripheral blood relies on markers identified in primary tumors or organs of origin. Given that no marker-gene is expressed in 100% of primary tumors and the fact that gene expression profiles of tumor cells change as a consequence of epithelial-mesenchymal transition (EMT) during tumor progression [62], a lot of circulating tumor cells could go undetected. As a result, indirect immunmediated and molecular detection techniques based on single or multimarker quantitative are inevitably inaccurate despite attempts to increase their sensitivity. Although the genetic heterogeneity of primary tumors has long been known, Klein et al. [63] showed that early disseminated cancer cells are genomically very unstable as well. Selection of clonally expanding cells leading to metastasis seems to occur after dissemination has taken place. The largest fraction of CTCs die or survive in a dormant state and will never develop into overt metastases. Therefore, adjuvant therapies are confronted with an extremely large reservoir of variant cells from which resistant tumor cells can be selected. Global gene expression profiles can be obtained from microarrays from the CTC-enriched portion of blood samples and can be compared with RNA extracted from the corresponding primary tumor, metastasis, or CTC-depleted portion of blood [64]. It could be argued that in the future, characterization of CTCs will guide the selection of cancer-specific genes expressed by the subpopulation of disseminated cancer cells responsible for metastasis.

**Future Perspectives**

An increasing body of evidence supports the hypothesis that the capacity of a tumor to grow and propagate is dependent on a small subset of cells with the capacity of self-renewal and differentiation, often termed “cancer stem cells” or “cancer-initiating cells” [65]. According to the cancer stem cell theory, tumor cells are heterogeneous, but only cancer stem cells are able to proliferate extensively and form new tumors [66]. Confirmation of the existence of these rare cells has been accumulating for hematopoietic malignancies, brain cancer [67], and solid organ malignancies including breast [68], prostate [69], colon [70, 71], and pancreatic cancer [72, 73]. For colon cancer, cancer stem cells are included in the high-density CD133+ population, which accounts for 2.5% [70] and 0.38% [71] of all tumor cells, respectively.

Both the concept of tumor cell heterogeneity and the cancer stem cell hypothesis put a caveat on the perception of metastasis as a stochastic phenomenon. Furthermore, all adjuvant therapies that rely on a single agent and do not target cancer stem cells will ultimately fail. In recent years, the interest in multicolor fluorescence cytometry has revived in an effort to study and isolate subpopulations of cancer cells with highest metastatic ability. In the future, quantification with high specificity of the “driving” cancer cell population in the peripheral blood of colon cancer patients may prove to have far better predictive value than quantifying all circulating cancer cells.

**CONCLUSIONS**

Although studies in the past have shown conflicting results, more and more evidence points toward a clinical relevance for quantification of solid tumor cells circulating in peripheral blood of colorectal cancer patients. Further research including standardization of study methodology is needed before circulating tumor cell quantification can be implemented into the routine clinical setting.

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**REFERENCES**


