MINIREVIEW—MOLECULAR PHARMACOLOGY IN CHINA

Clinical Applications of Circulating Tumor Cells in Pharmacotherapy: Challenges and Perspectives

Tong Wu, Bin Cheng, and Liwu Fu

State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangdong Esophageal Cancer Institute (T.W., L.F.); and Department of Oral Medicine, Guanghua School of Stomatology, Guangdong Provincial Key Laboratory of Stomatology, Sun Yat-sen University, Guangzhou, China (T.W., B.C.)

Received December 31, 2016; accepted March 22, 2017

ABSTRACT

Screening for circulating tumor cells (CTCs) has been identified as one approach to ultrasensitive liquid biopsy in real-time monitoring of cancer patients. The detection of CTCs in peripheral blood from cancer patients is promising as a diagnostic tool; however, the application of CTCs in therapeutic treatment still faces serious challenges with respect to specificity and sensitivity. Here, we review the significant roles of CTCs in metastasis and the strengths and weaknesses of the currently available methods for CTC detection and characterization. Moreover, we discuss the clinical application of CTCs as markers for patient prognosis, and we specifically focus on the application of CTCs as indicators in cancer pharmacotherapy. Characterization of the detected CTCs will provide new biologic perspectives and clinical applications for the treatment of cancer patients with metastasis.

Introduction

A fraction of the cancer cells that disseminate from primary tumors and migrate to distant sites will result in the formation of a lethal metastatic tumor (Hanahan and Weinberg, 2011). Migrating tumor cells found in the peripheral bloodstream are called circulating tumor cells (CTCs), while their counterparts found in bone marrow are called disseminated tumor cells (Alix-Panabieres and Pantel, 2016). CTCs have been regarded as a critical stage in the development of metastasis, in that they contain genetic and molecular information about the cancer as well as its evolutionary adaptation to prior therapies (Alix-Panabières and Pantel, 2016). However, CTCs have also been detected in the blood circulation of healthy volunteers, in patients with benign diseases of the lung, colon, pancreas, and breast (Franken et al., 2012; Pantel et al., 2012; Ilie et al., 2014; Markou et al., 2014; Alva et al., 2015; Cauley et al., 2015; Liu et al., 2015).

CTCs have paved new diagnostic avenues in liquid biopsy diagnostics, especially for tumors that are not easy to biopsy and for metastatic lesions (Azarin et al., 2015). Early detection of cancer metastasis is always difficult, not to mention its prevention or cure. With its noninvasive nature and real-time advantage, cancer screening for CTCs can be applied to populations at higher risk. Therefore, oncologists place high hopes on CTC-based screening methods, which have been found to be more sensitive than current imaging methods such as positron emission tomography (Hegemann et al., 2016). The consistency between CTCs and their primary tumors is encouraging, and may provide an excellent opportunity for clinicians to examine mutations of key genes that are not detected through traditional blood-based assays (Nagrath et al., 2016). In the era of precision medicine in cancer therapy, systemic monitoring of response to anticancer therapies is a key step toward providing personalized care. An increasing body of evidence indicates that, in the course of treatment, assessment of the molecular characteristics of a progressive disease is more significant than depending on the results obtained for primary tumor samples, which do not reflect the evolution of the tumor (Alix-Panabières and Pantel, 2016). Comprehensive information regarding whole disease processes obtained from studies on CTCs has not only revealed the underlying mechanisms of tumorigenesis and metastasis, but has also provided noninvasive methods for cancer diagnosis, prognosis, and pharmacotherapy monitoring (Masuda et al., 2016).

Recent research has demonstrated that CTCs, an integral part of the liquid biopsy, have great potential to change the
status quo of anticancer therapy; however, the approach remains technically challenging. Following a short discussion of the significant roles of CTCs in cancer metastasis and the currently available methods for CTC detection and molecular characterization, this review will focus on the clinical applications of CTCs as markers for prognosis prediction in cancer patients and as indicators in cancer pharmacotherapy (Fig. 1).

CTCs and Cancer Metastasis

The malignant form of cancer and the cause of more than 90% of cancer-associated mortality cases is metastasis, which is characterized by the ability of cancer cells to invade the surrounding tissue, disseminate throughout the body, and establish secondary tumors in distant organs (Gupta and Massagué, 2006). As shown in Fig. 2, the sequential metastasis process is first initiated by the loss of adhesion of tumor cells in the primary site and their migration out of the primary tumor. Second, the tumor cells attach to blood vessels and invade the blood or lymphatic circulation in a process called intravasation (Fidler, 2003). As the tumor cells circulate to the secondary site, the tumor cells intrude blood vessels, adhere to the target organ endothelium, and migrate into the parenchyma; this is called extravasation (Klein, 2009; Chaffer and Weinberg, 2011; Sosa et al., 2014). Therefore, the existence of CTCs has been recognized as an important intermediate step in cancer metastasis. CTCs represent a stem-like subpopulation of cells that are capable of immigration and tumor initiation (Al-Hajj et al., 2003; Ricci-Vitiani et al., 2007; Stewart et al., 2011). During the metastasis process, many cell surface markers of CTCs undergo change. The activation of an epithelial-to-mesenchymal transition (EMT) facilitates tumor cell invasion and dissemination during intravasation, while its reverse process, a mesenchymal-to-epithelial transition, is believed to support extravasation once cancer cells have arrived in distant organs (Acloque et al., 2009; Thiery et al., 2009; Nieto, 2013). It has been speculated that the entire process in which CTCs seed metastasis occurs with extremely low efficiency; only 0.01% of all CTCs can survive and form micrometastases in distant organs (Luzzi et al., 1998). Even if CTCs succeed in intravasation, most of them cannot survive the adverse environment in the bloodstream and eventually die from anoikis. Therefore, CTCs sometimes aggregate to form microemboli (circulating tumor microemboli), which may be advantageous in the survival of tumor cells and enhance the viability and motility of CTCs (Krebs et al., 2014).

For several decades, the lack of relevant models for metastasis research has extensively limited further investigation. CTCs are the true link between primary and metastatic tumors, and thus create a new opportunity for investigators to explore valuable features of both primary and metastatic sites, as well as specific details of the processes of intravasation, migration, and extravasation.

CTC Detection

As previously mentioned, after CTCs are released from the main and/or metastatic tumor site into the blood circulation, the conditions in the blood are harsh for epithelial tumor cells, and the survival time of CTCs is extremely short (half-life: 1–2.4 hours) (Meng et al., 2004). Apoptotic and fragmented CTCs are frequently detected in the peripheral bloodstream of cancer patients (Larson et al., 2004). Therefore, a pivotal challenge in the clinical application of CTCs is the capability of the current CTC technology to efficiently capture the extremely rare CTC population from blood samples of patients for subsequent processing (Nelson, 2010).

In the past decade, one of the most widely used strategies to detect CTCs has been the use of epithelial markers such as cytokeratins (CKs) and EpCAM, which are not detected in the surrounding mesenchymal blood cells. CellSearch, the only U.S. Food and Drug Administration approved CTC technology to monitor metastatic breast cancer patients, is a case in point (Ferreira et al., 2016). The CellSearch system uses ferrofluid functionalized nanoparticles containing the EpCAM antibody for magnetic separation of EpCAM positive cells after blood centrifugation for the enrichment of CTCs. The cells are then selected by immunostaining for the expression of CK8, CK18, and CK19, as well as for negative staining for CD45 (Riethdorf et al., 2007).

However, further research has demonstrated that epithelial tumor cells are likely to undergo EMT, which results in
CTCs as Prognostic Markers in Cancer

Currently, CTC enumeration is widely used as a prognostic index for patient overall survival rate. A cutoff value of ≥5 or ≥3 CTCs in 7.5 ml blood has been proven to be a poor prognostic index in several cancers, including breast cancer (Zhang et al., 2012), colorectal cancer (Cohen et al., 2008), prostate cancer (de Bono et al., 2008), lung cancer (Krebs et al., 2011), bladder cancer (Gazzaniga et al., 2014), liver cancer (Schulze et al., 2013), esophageal cancer (Vashist et al., 2012), melanoma (Rao et al., 2011), head and neck carcinoma (Nichols et al., 2012), and pancreatic cancer (Han et al., 2014).

The association between detection of CTCs and clinical outcome has been most widely studied in breast cancer. For example, CTCs were analyzed in a pool of 2026 patients with early stage breast cancer before pharmacotherapy and 1492 patients after pharmacotherapy using the CellSearch System (Rack et al., 2014). In the pre-pharmacotherapy group, CTCs were found in 21.5% of patients, in which 19.6% were lymph node negative and 22.4% were node positive. No correlation was found between CTCs and tumor size, grading, or hormone receptor status. CTCs were detected in 22.1% of 1492 patients after chemotherapy. The presence of CTCs was associated with poor rates of disease-free, distant disease-free, breast cancer-specific, and overall survival. CTCs were identified as an independent prognostic index for disease-free survival in multivariable analysis. Patients with more than five CTCs per 30 ml blood had the worst prognosis. These results from a large-scale trial of patients with breast cancer suggested that CTCs have prognostic value (Rack et al., 2014).

Of note, in these reports indicating that CTCs can be used as a reliable early index of disease progression and survival compared with traditional methods, a significant proportion of patients with obvious distant metastases were negative for CTCs. This result implied that CTCs undergoing EMT transformation can be missed by epithelial marker–based detection methods, such as the CellSearch system. Therefore, large-scale multicenter trials with improved CTC detection techniques and well-defined endpoints are needed to support the clinical utility of CTC detection in cancer patients.

CTCs as Indicators in Pharmacotherapy

CTCs may be disseminated from the primary tumor or from a number of metastatic sites. Therefore, CTCs offer a wealth of genetic and molecular information concerning the cancer at the protein, RNA, and genome levels (Meng et al., 2004). In addition to CTC detection, significant effort has been made toward CTC characterization. In the era of precision medicine of cancer therapy, identification of CTCs expressing certain markers can be used to specifically monitor cancer therapy.
<table>
<thead>
<tr>
<th>Enrichment</th>
<th>Selection Criteria</th>
<th>Assay System</th>
<th>Tumor Origin</th>
<th>Key Feature</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoaffinity positive</td>
<td>EpCAM</td>
<td>CellSearch</td>
<td>Colorectal, breast, prostate, ovarian, lung</td>
<td>FDA approved</td>
<td>Ohnaga et al. (2016); Grisanti et al. (2016); Shaw et al. (2017); Sholl et al. (2016); Van Berckelaer et al. (2016)</td>
</tr>
<tr>
<td>MagSweeper</td>
<td>Breast, prostate</td>
<td></td>
<td></td>
<td>High purity</td>
<td>Cann et al. (2012); Deng et al. (2014)</td>
</tr>
<tr>
<td>CTC-Chip</td>
<td>Breast, esophageal, prostate, lung</td>
<td></td>
<td></td>
<td>Microport array optimized for high detection rate</td>
<td>Jiang et al. (2015); Khamenehfar et al. (2015); Ohnaga et al. (2016); Sequist et al. (2009); Sheng et al. (2014)</td>
</tr>
<tr>
<td>GEM</td>
<td>Pancreatic</td>
<td></td>
<td></td>
<td>Microwortices sample mixture</td>
<td></td>
</tr>
<tr>
<td>Antibody cocktail</td>
<td>AdnaTest</td>
<td></td>
<td></td>
<td>Multiple cancer marker measured by RT-PCR</td>
<td>Blasi et al. (2016); Bredemeier et al. (2016); Capoun et al. (2016); Gorges et al. (2016); Winer-Jones et al. (2014)</td>
</tr>
<tr>
<td>LiquidBiopsy</td>
<td>Breast</td>
<td></td>
<td></td>
<td>Automated, sheath flow minimized nonspecific binding continuous flow</td>
<td></td>
</tr>
<tr>
<td>Immunoaffinity negative</td>
<td>CD45, CD66b, size</td>
<td>CTC-iChip</td>
<td>Lung, prostate, pancreas, breast, melanoma</td>
<td>Size-based separation</td>
<td>Karabacak et al. (2014); Ozkumur et al. (2013)</td>
</tr>
<tr>
<td>Density gradient centrifugation</td>
<td>Density</td>
<td>Accucyte Enrichment and CyteSealer</td>
<td>Lung, prostate, breast</td>
<td>Sequential density fractionation enriches target cells</td>
<td>Campton et al. (2015)</td>
</tr>
<tr>
<td>Microfiltration in two and three dimensions</td>
<td>Size, deformability</td>
<td>ISET</td>
<td>Colorectal, melanoma, esophageal, pancreatic, lung</td>
<td>Sensitive threshold of one carcinoma cell per milliliter of blood</td>
<td>Abdallah et al. (2016); Li et al. (2015); Long et al. (2016); Pailier et al. (2015)</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>Electrical Signature</td>
<td>ApoStream</td>
<td>Lung, breast, ovarian</td>
<td>Continuous flow; captures viable cells</td>
<td>Gupta et al. (2012); OShanessy et al. (2016)</td>
</tr>
<tr>
<td>Inertial focusing</td>
<td>Size</td>
<td>Vortex</td>
<td>Lung, breast</td>
<td>Microfluidic technology for the label-free, size-based enrichment and concentration of rare cells</td>
<td>Che et al. (2015); Dhar et al. (2015); Sollier et al. (2014)</td>
</tr>
<tr>
<td>Acoustophoresis</td>
<td>Size</td>
<td>Acoustophoresis Chip</td>
<td>Breast</td>
<td>Acoustic prealignment and separation</td>
<td>Antfolk et al. (2015)</td>
</tr>
<tr>
<td>Direct imaging modalities</td>
<td>CK, CD45, DRAQ5</td>
<td>Image Stream</td>
<td>Pancreaticobiliary, esophageal, hepatocellular, thyroid, ovarian</td>
<td>Hybrid of flow cytometry and fluorescence microscopy</td>
<td>Catenacci et al. (2015); Dent et al. (2016); López-Riquelme et al. (2013); Starzyńska et al. (2013); Alix-Panabères et al. (2012); Denève et al. (2013); Ramirez et al. (2014)</td>
</tr>
<tr>
<td>Functional assays</td>
<td>Protein secretion</td>
<td>EPISPOT</td>
<td>Breast, colorectal, colon</td>
<td>Detection of viable epithelial secreting cells</td>
<td></td>
</tr>
<tr>
<td>Cell adhesion matrix</td>
<td>Vita-Assay</td>
<td></td>
<td>Prostate, lung, pancreatic</td>
<td>Detection of CTCs with the invasive phenotype in blood</td>
<td>Friedlander and Fong (2014); Tulley et al. (2016)</td>
</tr>
</tbody>
</table>

FDA, Food and Drug Administration; RT-PCR, reverse-transcription polymerase chain reaction.
CTCs as Markers for Targeted Therapy Selection. Molecular alterations in CTCs have proven to be highly consistent in primary tumors, thus providing robust evidence for the clinical application of targeted therapies in cancer. Several studies have suggested that CTCs can be used as an index in therapy selection, and that they can also be used in real-time biopsies to reflect the effect of a particular therapy. For example, BRAF mutations between primary tumors and metastases have been found in patients; these mutations mediate tumor proliferation through activation of the RAF-MEK-ERK pathway (Lin et al., 2011). Therefore, the BRAF mutation status in CTCs collected from patients with metastatic melanoma is a pivotal index in selecting targeted therapies such as vemurafenib and dabrafenib (Jang and Atkins, 2014; Reid et al., 2015). Another case in point is epidermal growth factor receptor (EGFR) mutations in lung cancer. A group of pulmonary adenocarcinoma that has activating EGFR mutations is exclusively sensitive to EGFR tyrosine kinase inhibitors (Mok et al., 2009). Therefore, EGFR mutations in CTCs are clinical biomarkers in the categorization of pharmacotherapy targets in metastatic lung cancer with respect to treatment with erlotinib, afatinib, and osimertinib (Breitenbucher et al., 2014; Kuwano et al., 2016). In addition to melanoma and lung cancer, therapeutic targets were identified in breast cancer. The phosphatidylinositide 3-kinase/protein kinase B/mammalian target of the rapamycin pathway is frequently altered in cancer. Phosphatidylinositide 3-kinase is a cell membrane signal transduction molecule that supports cell survival and growth, making it a popular therapeutic target (Wong et al., 2010; Akinleye et al., 2013). PI3KCA mutations were identified in CTCs from metastasis breast cancer patients by CellSearch enrichment, DNA extraction, and whole genome amplification (Schneck et al., 2013); therefore, agents that target this pathway, such as everolimus and temsirolimus, are promising therapeutic options (Johnston, 2015).

Another application of CTCs is in the detection of various biomarkers expressed in advanced disease that reflect the progression of the cancer. The hormone receptor status is one of the most well-established predictors in endocrine adjuvant or palliative therapy of primary and metastatic breast cancer. However, the hormone receptor status changes during the course of disease progression. Variations in the expression of estrogen receptor and human epidermal growth factor receptor 2 can occur in advanced breast cancer, and has been readily detected in CTCs. Monitoring these changes is helpful when selecting chemotherapies, especially those targeting the human epidermal growth factor receptor, such as trastuzumab, lapatinib, pertuzumab, and trastuzumab-entansine (Thompson et al., 2010; Aktas et al., 2011; Turner and Di Leo, 2013; Hernández-Blanquissett et al., 2016). In addition, several therapeutic targets such as anaplastic lymphoma kinase (Ilie et al., 2012; Paillier et al., 2013), PD-L1 (Jing et al., 2016), and RAS (Karandish and Mallik, 2016), were also detected in CTCs collected from breast, colorectal, prostate, and ovarian cancer patients (Fig. 3).

CTCs as Markers of Treatment Resistance. It has been reported that in both early and metastatic cancers the presence of CTCs following treatment can act as a predictive index of the possibility of disease recurrence (Xenidis et al., 2007; Alix-Panabières and Pantel, 2013). These persisting CTCs are resistant to treatment and can thus be involved in cancer progression. Therefore, there is an urgent need to identify effective therapies in patients with therapy-resistant CTCs. Several studies have been reported based on this strategy. For example, platinum resistance is one of the most recognized clinical challenges for ovarian cancer pharmacotherapy. While detection of the ERCC1 protein in the primary tumor by immunohistochemistry is inaccurate in predicting platinum resistance, the presence of ERCC1+ CTCs in blood can be used as a diagnostic biomarker in ovarian cancer to predict platinum resistance (Kuhlmann et al., 2014). In metastatic castration-resistant prostate cancer, the presence of androgen receptor splice variant 7 (AR-V7) in CTCs is associated with resistance to enzalutamide and abiraterone, but not to taxanes (Antonarakis et al., 2014, 2015). In AR-V7-positive patients, taxanes are more efficacious than enzalutamide or abiraterone therapy in AR-V7-positive tumors, while in AR-V7-negative patients, taxanes, enzalutamide, and abiraterone have quite similar efficacies (Antonarakis et al., 2015). Therefore, AR-V7 expressed in CTCs may serve as a biomarker for castration-resistant prostate cancer treatment selection (Onstenk et al., 2015). These results add to existing evidence that CTCs are a valuable tool that can be used to optimize personalized cancer treatments and improve the prognosis in therapy-resistant patients.

CTCs as a Biomarker for Treatment Sensitivity. Increasing evidence points to the significance of evaluating the molecular features of advancing disease during therapy instead of depending on primary tumor samples, which are unable to reflect the progression of a tumor and target the associated features (Alix-Panabières and Pantel, 2016). Considering the easy availability of blood, it has been suggested that CTCs can serve as a real-time liquid biopsy to provide information on a current disease without invasive biopsy (Lianidou and Markou, 2011).

CTC enumeration is one of the most widely used criteria to monitor systemic anticancer therapy. The significance of CTC enumeration in monitoring anticancer therapy has been demonstrated in metastatic breast cancer patients receiving...
first-line chemotherapy. In the SWOG 0500 (NCT00382018) clinical trial (http://swog.org/visitors/ViewProtocolDetails.aspx? ProtocolID=2046), patients with metastatic breast cancer had CTC enumeration before cycles 1 and 2 with or without targeted therapy in combination with first-line chemotherapy. Patients with sustained increases in the CTC number (≥5 CTCs/7.5 ml) after one cycle of treatment were regarded as a higher risk group for early cancer progression. These patients were randomly designated into a continued first-line pharmacotherapy group or another treatment group before radiologic evaluation of progression (Bidad et al., 2016). For patients with continued increases in CTC numbers after first-line chemotherapy, a more effective treatment than standard chemotherapy is needed (Smerage et al., 2014). Several multicenter clinical trials testing anticancer therapy monitoring based on CTCs are still in progress, including the STIC CTC METABREAST clinical trial in France (NCT01710605) (https://clinicaltrials.gov/ct2/show/NCT01710605). In this trial, breast cancer patients with more than five CTC counts in 7.5 ml blood received chemotherapy, while patients with no more than five CTCs in 7.5 ml blood received endocrine therapy as the first-line treatment (Liangidou and Markou, 2011). In a phase II trial of erlotinib and pertuzumab in members of the METABREAST clinical trial in France (NCT01710605), patients with metastatic castration-resistant prostate cancer. JAMA Oncol 1:582–591.


