Circulating Tumor Cells

Subjects: Allergy
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Many different therapies are applied to fight tumor disease. Blood-based biosources, like circulating tumor cells (CTCs), offer the opportunity to monitor the healing progression and the real-time response to the therapy.

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1. Introduction

Cancer is caused by multiple molecular alterations in normal host cells that act together to drive uncontrolled cell self-renewal, growth and invasion, and lead to malignant transformation and progression. The majority of cancer-associated deaths (approximately 90%) are induced by metastatic disease rather than the primary cancer [1]. The early detection of cancer and subsequent noninvasive tumor profiling and monitoring should be enabled in every cancer patient. Thus, there is an unmet clinical need for biomarkers to fulfill the claim in precision oncology.

Blood-based biosources such as circulating tumor cells (CTCs), cell-free DNA (cfDNA), tumor-educated platelets and cell-free nucleic acids (circulating tumor DNA, long non-coding RNA, messenger RNA and microRNA) offer this opportunity. These biomarkers, summarized as liquid biopsy (LB), could provide information on urgent cancer characteristics [2]. CTCs detach from primary or metastatic tumors to enter the bloodstream, from which a small CTC population has the ability to metastasize to multiple organs [3]. In addition, CTCs are genetically unstable, evading immune defenses and modification metabolism [3][4]. These characteristics reflect the dynamic and heterogeneous phenotype of CTCs. Furthermore, at present, we know that cancer cells survive after infiltrating distant organs and can be present for years in the bone marrow as disseminated tumor cells (DTCs), which are correlated with an increased risk of eventual clinical recurrence [5].

The concentration of CTCs in the blood is very low, and a single CTC is in the background of millions of blood cells. Nonetheless, CTCs could serve as a comprehensive window into metastatic disease for the real-time monitoring of therapy responses.

LB in the form of CTCs received tremendous attention following approval of the automated CellSearch® system (Menarini Silicon Biosystems Inc, Huntington Valley, USA) by the Food and Drug Administration (FDA). Thus, the importance of CTC enumeration as a surrogate marker for survival benefits in breast and prostate cancer patients was commenced [6][7].

The clinical utility and reliable information of CTCs as useful biomarkers must still be demonstrated in the standard care of cancer therapy. In the latest guidelines (version 3) the Prostate Cancer Clinical Trials Working Group (PCWG) determined that for the outcome assessment of patients enrolled in clinical trials, the incorporation of CTC enumeration (using CellSearch platform) must be the endpoint [8]. This decision illustrates and emphasizes the importance of the serial biological profiling of cancer. Moreover, it promotes CTCs in the field of personalized cancer treatment, supplying unique information on individual cancer-associated variations in tumor burden.

2. Position of Circulating Tumor Cells in the Clinical Routine in Prostate Cancer and Breast Cancer Patients

Cancer cells can enter and are motile in blood circulation long before the tumor diagnosed. They offer a possibility for an early cancer diagnosis than standard diagnostics tools of imaging or biomarkers. The CTC analysis can provide insight into personalized cancer characteristics.

CTCs are relatively rare cells with a heterogenetic phenotype and are difficult to capture. At present, there are several platforms that can be used to capture or characterize CTCs. All of these platforms have advantages and disadvantages. The user must be able to decide which is the best and most suitable method for individual cancer patients. However, it is possible that in metastatic or late stage patients, no CTCs are detected. One reason for such conditions, could be the necrotic changes, or reduced tumor vascularization or extreme heterogenic CTC population. Another reason is the

variation of the CTC frequency in a single blood sample, which also includes the short half-life of CTC and possible circadian rhythms of CTCs. Furthermore, it must be considered that phenotypic changes in CTC, in terms of epithelial-to-mesenchymal transition (EMT), reflects features of and results in downregulation of cell surface marker EpCAM.

The EMT process is also implicated in the generation of cancer stem cells (CSC), which are cells with abilities to self-renewal [9]. Such changes increase aggressiveness of the tumor cells, provoke their dissemination from the tumor and induce metastases. Detection of CSC as subpopulation CTCs and EMT-CTCs by patients would be crucial information for the treatment and potential therapy resistance.

A more precise analysis of CTCs would be applying the markers like Vimentin (EMT) or CD44 (CSC) at any level (DNA, RNA, protein) in regard to their clinical utility. The broader knowledge on intratumor heterogeneity and dynamic genetic and physiological changes in CTCs undergoing EMT or stem cell CTCs enforces continuous widening of the spectrum of specific markers to be analyzed.

The tumor-released cells can circulate cell-clusters composed of different CTC subpopulation. Only a few of the reviewed trials analyzed CTC-clusters additional to CTCs. The presence of CTC-clusters supported the previous diagnosis obtained with CTCs [10][11].

In our opinion, the crucial point of the efficient CTC analysis, enabling to include them into diagnostics, is their isolation. The combination of the EpCAM-based CellSearch® system with antibody-independent CTC isolation platforms in one blood sample extends the best suitable CTC isolation method. This enables the isolation of all subpopulation of CTCs including CTC, EMT-CTC, CSC and can provide a precise outcome. Approbation of such a system or platform as an additional or supporting diagnostic tool would make the therapy more precise.

Such an analysis could be helpful to avoid the low response rate to therapy. A good example is the detection of the HER2 status in circulating breast cancer cells, which could be opposite to that in the primary tumor [12]. A combined analysis of additional factors, as described by Paoletti et al. [13] (multiparameter CTC-Endocrine Therapy Index (CTC-ETI)), supplies more information and may predict resistance to endocrine therapy. In preclinical studies, researchers analyzed the combined results of the enumeration and expression of ER, HER2 and Ki-67 in MBC patients and demonstrated strong analytical validity of the technique through intrapatient heterogeneity [14].

The molecular characterization of AR mRNA in CTCs and the detection of AR-V7 in CTCs can be used as a tool to guide treatment decisions for men with advanced prostate cancer [15][16]. The benefits for patients are that they are protected against the unnecessary side effects of ineffective treatments.

During their investigations, Smerage et al. [17] revealed that in patients with high numbers of CTCs after treatment, the number of cells did not change; thus, the number of apoptotic cells increased. High levels of M30-CTC (apoptotic cells) correlate with a poor prognosis, whereas high levels of CTC-Bcl-2 (anti-apoptotic cells) are associated with a good prognosis [17]. This is a very important observation, as the initiation of apoptosis in cells as a reaction to environmental stress leads to many morphological and biochemical changes, and probably to the production and secretion of substances that can be spread via the bloodstream to other organs, causing damage [18].

The signals from the tumor microenvironment (TME) and the microenvironment of CTC population can modify the protein pattern of disseminated cells, preparing it to create metastasis. One of the proteins induced by TME but also by chemotherapy is prostaglandin (PG)-endoperoxide synthase 2 (COX-2), which finally promotes the carcinogenesis and the rate of cancer recurrence, reducing the survival rate. COX-2 is implicated in the suppression of the apoptosis causing the resistance of tumor cells. The downstream signaling protein of the COX-2 is, among others, the Bcl-2, the anti-apoptotic marker increased in CTCs analyzed by Smerage et al. [19] (reviewed in [20]). Since both the positive and the negative signals may be initiated by the therapy, the monitoring of the treatment is crucial. LB, as the CTCs, is an excellent tool to analyze the changes during the long healing process. Although this hypothesis needs further investigation, if it is correct, it could bear significant consequences. The critical point of preanalytical variables on LB in prostate cancer was also discussed in the plenary session of the ACTC 2019 meeting [21]; however, this issue is also applicable for other cancer types, such as breast cancer. Howerd Scher pointed out, in this session, the importance of introducing robust quality controls in all steps, such as pre-analytical procedures, and sample collection and processing, as well as analytical steps like molecular assay specification and bioinformatics algorithm, and data reporting. This standardization is absolutely necessary for its application into clinical routine. Alix-Panabieres [22] summarized in her research, the need for more intervention studies on the implementation of CTCs in the clinic. All abstracts at the 4th ACTC meeting described diverse proofs of concept in CTC isolation and characterization, and confirmed the heterogeneity in this field.

Cabel et al. [23] summarized an analysis of the utility of CTCs in clinical trials. They noted three main concepts of the studies: (1) CTCs serve as surrogate tumor material, (2) CTC enumeration can be used to monitor therapy, and (3) specific biological features of CTCs and their relation to metastatic spread. This conclusion is still relevant. The clinical validity of CTC enumeration by the CellSearch® system is very high; however, its utility is still not standardized in the clinic and requires further investigations. Moreover, the clinical relevance of CTC characterization indicates the current therapeutic target HER2/ER in breast cancer patients and AR-V targets elucidates the resistance mechanism for prostate cancer patients. Even if there is a great need to identify predictive markers for therapy, it remains a great challenge to implement CTCs as a robust standard tool in the clinic.

References

1. Lambert, A.W.; Pattabiraman, D.R.; Weinberg, R.A. Emerging biological principles of metastasis. Cell 2017, 168, 670–691.
2. Alix-Panabieres, C.; Pantel, K. Circulating tumor cells: Liquid biopsy of cancer. Clin. Chem. 2013, 59, 110–118.
3. Massague, J.; Obenauf, A.C. Metastatic colonization by circulating tumour cells. Nature 2016, 529, 298–306.
4. Welch, D.R.; Hurst, D.R. Defining the hallmarks of metastasis. Cancer Res. 2019, 79, 3011–3027.
5. Braun, S.; Vogl, F.D.; Naume, B.; Janni, W.; Osborne, M.P.; Coombes, R.C.; Schlimok, G.; Diel, I.J.; Gerber, B.; Gebauer, G.; et al. A pooled analysis of bone marrow micrometastasis in breast cancer. N. Engl. J. Med. 2005, 353, 793–802.
6. De Bono, J.S.; Scher, H.I.; Montgomery, R.B.; Parker, C.; Miller, M.C.; Tissing, H.; Doyle, G.V.; Terstappen, L.W.; Pienta, K.J.; Raghavan, D. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin. Cancer Res. 2008, 14, 6302–6309.
7. Cristofanilli, M.; Budd, G.T.; Ellis, M.J.; Stopeck, A.; Matera, J.; Miller, M.C.; Reuben, J.M.; Doyle, G.V.; Allard, W.J.; Terstappen, L.W.; et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N. Engl. J. Med. 2004, 351, 781–791.
8. Scher, H.I.; Morris, M.J.; Stadler, W.M.; Higano, C.; Basch, E.; Fizazi, K.; Antonarakis, E.S.; Beer, T.M.; Carducci, M.A.; Chi, K.N.; et al. Trial design and objectives for castration-resistant prostate cancer: Updated recommendations from the prostate cancer clinical trials working group 3. J. Clin. Oncol. 2016, 34, 1402–1418.
9. Scheel, C.; Weinberg, R.A. Cancer stem cells and epithelial-mesenchymal transition: Concepts and molecular links. Semin. Cancer Biol. 2012, 22, 396–403.
10. Autio, K.A.; Dreicer, R.; Anderson, J.; Garcia, J.A.; Alva, A.; Hart, L.L.; Milowsky, M.I.; Posadas, E.M.; Ryan, C.J.; Graf, R.P.; et al. Safety and Efficacy of BIND-014, a Docetaxel Nanoparticle Targeting Prostate-Specific Membrane Antigen for Patients with Metastatic Castration-Resistant Prostate Cancer: A Phase 2 Clinical Trial. JAMA Oncol. 2018, 4, 1344–1351.
11. Larsson, A.M.; Jansson, S.; Bendahl, P.O.; Jörgensen, C.L.T.; Loman, N.; Graffman, C.; Lundgren, L.; Aaltonen, K.; Rydén, L. Longitudinal enumeration and cluster evaluation of circulating tumor cells improve prognostication for patients with newly diagnosed metastatic breast cancer in a prospective observational trial. Breast Cancer Res. 2018, 20, 48.
12. Fehm, T.; Müller, V.; Aktas, B.; Janni, W.; Schneeweiss, A.; Stickeler, E.; Lattrich, C.; Löhberg, C.R.; Solomayer, E.; Rack, B.; et al. HER2 status of circulating tumor cells in patients with metastatic breast cancer: A prospective, multicenter trial. Breast Cancer Res. Treat. 2010, 124, 403–412.
13. Paoletti, C.; Li, Y.; Muñiz, M.C.; Kidwell, K.M.; Aung, K.; Thomas, D.G.; Brown, M.E.; Abramson, V.G.; Irvin, W.J., Jr.; Lin, N.U.; et al. Significance of circulating tumor cells in metastatic triple-negative breast cancer patients within a randomized, phase II trial: TBCRC 019. Clin. Cancer Res. 2015, 21, 2771–2779.
14. Paoletti, C.; Schiavon, G.; Dolce, E.M.; Darga, E.P.; Carr, T.H.; Geradts, J.; Hoch, M.; Klinowska, T.; Lindemann, J.; Marshall, G.; et al. Circulating biomarkers and resistance to endocrine therapy in metastatic breast cancers: Correlative results from AZD9496 oral SERD phase I trial. Clin. Cancer Res. 2018, 24, 5860–5872.
15. Antonarakis, E.S.; Lu, C.; Luber, B.; Wang, H.; Chen, Y.; Nakazawa, M.; Nadal, R.; Paller, C.J.; Denmeade, S.R.; Carducci, M.A.; et al. Androgen Receptor Splice Variant 7 and Efficacy of Taxane Chemotherapy in Patients with Metastatic Castration-Resistant Prostate Cancer. JAMA Oncol. 2015, 1, 582–591.
16. Armstrong, A.J.; Halabi, S.; Luo, J.; Nanus, D.M.; Giannakakou, P.; Szmulewitz, R.Z.; Danila, D.C.; Healy, P.; Anand, M.; Rothwell, C.J.; et al. Prospective Multicenter Validation of Androgen Receptor Splice Variant 7 and Hormone

Therapy Resistance in High-Risk Castration-Resistant Prostate Cancer: The PROPHECY Study. J. Clin. Oncol. 2019, 37, 1120–1129.

17. Smerage, J.B.; Budd, G.T.; Doyle, G.V.; Brown, M.; Paoletti, C.; Muniz, M.; Miller, M.C.; Repollet, M.I.; Chianese, D.A.; Connelly, M.C.; et al. Monitoring apoptosis and Bcl-2 on circulating tumor cells in patients with metastatic breast cancer. Mol. Oncol. 2013, 7, 680–692.
18. Paoletti, C.; Muniz, M.C.; Thomas, D.G.; Griffith, K.A.; Kidwell, K.M.; Tokudome, N.; Brown, M.E.; Aung, K.; Miller, M.C.; Blossom, D.L.; et al. Development of circulating tumor cell-endocrine therapy index in patients with hormone receptor-positive breast cancer. Clin. Cancer Res. 2015, 21, 2487–2498.
19. Onstenk, W.; de Klaver, W.; de Wit, R.; Lolkema, M.; Foekens, J.; Sleijfer, S. The use of circulating tumor cells in guiding treatment decisions for patients with metastatic castration-resistant prostate cancer. Cancer Treat. Rev. 2016, 46, 42–50.
20. Hashemi Goradel, N.; Najafi, M.; Salehi, E.; Farhood, B.; Mortezaee, K. Cyclooxygenase-2 in cancer: A review. J. Cell Physiol. 2019, 234, 5683–5699.
21. Lianidou, E.; Pantel, K. Hellenic society of liquid biopsy. In Proceedings of the 4th ACTC | Advances in Circulating Tumor Cells, Corfu, Greece, 2–5 October 2019.
22. Alix-Panabieres, C. The future of liquid biopsy. Nature 2020, 579, S9.
23. Cabel, L.; Proudhon, C.; Gortais, H.; Loirat, D.; Coussy, F.; Pierga, J.Y.; Bidard, F.C. Circulating tumor cells: Clinical validity and utility. Int. J. Clin. Oncol. 2017, 22, 421–430.
