Detection methods of circulating tumor cells

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Early during the formation and growth of a primary epithelial tumor, cells disseminate through the bloodstream to distant organs. These circulating tumor cells (CTCs) can be enriched and detected via different technologies and CTC analyses are considered as a *real-time "liquid biopsy"* in cancer patients. This biopsy allows the characterization of specific sub-populations of CTCs and may revolutionize cancer detection and management. This editorial highlights the current strategies used for enrichment and detection of CTCs.

CTC detection holds great promise, and many exciting technologies have been developed over the past years. However, detecting CTCs remains technically very challenging. As CTCs occur at very low concentrations (1 CTC in the background of millions of normal hematopoietic cells), their identification and characterization require extremely sensitive and specific analytical methods, which are usually a combination of enrichment and detection procedures (1).

CTC enrichment includes a large panel of technologies based on the different properties of CTCs that distinguish them from the surrounding normal blood cells, including *physical properties* (size, density, electric charges, deformability) and *biological properties* (surface protein expression, mostly EpCAM expression). To increase the yield of CTCs, new technologies based on the combination of different CTC properties have been even developed. After enrichment, the CTC fraction usually still contains a substantial number of leukocytes, and CTCs need to be, therefore, identified by a method that can distinguish tumor cells from normal blood cells at the single cell level. Among the ***protein-based strategies***, the CellSearch® system (FDA-USA cleared) and many other CTC assays use the same identification step: cells are fluorescently stained for cytokeratins (CK; positive marker), the common leukocyte antigen CD45 (negative marker), and a nuclear dye (DAPI); CTCs are identified as $CK^+/CD45^-/DAPI^+$ cells. One key question is whether the detected CTCs are viable or apoptotic because only functional cells should be able to contribute to metastasis formation. In order to detect only living CTCs, the functional EPISPOT assay (for EPithelial ImmunoSPOT), which can be added to any kind of enrichment step, was introduced for CTC detection. Avoiding direct contact with the target cells, this technique assesses the presence of CTCs based on secreted, shed, or released proteins during 48 h of short-term culture (2-4). These last 4 years, there was an important focus on the development of microfluidic devices ("CTC-chips"), which can handle very small blood volumes. The first CTC-chip consisted of an array of anti-EpCAM antibody-coated microposts and has been further developed into a herringbone structure (5). Since 2008, new CTC-chips have been improved [i.e., Ephesia-chip (6), chip-based micro-Hall detector (7), CTC-chip for CK^+ & CK^- CTCs (8)]. Moreover, among ***mRNA-based strategies***, assays targeting specific mRNAs are the most widely used alternative to immunological assays to identify CTCs. In breast cancer, the CK19 mRNA has been most frequently used in clinical studies (9). Many transcripts (e.g., encoding CK18, CK19, CK20, MUC1, PSA, and CEA), however, are also expressed at low levels in normal blood and BM cells (9-10), and quantitative RT-PCR assays with validated cut-off values are therefore required to overcome this problem. Moreover, gene transcription might be modified in CTCs, which argues in favour of multimarker RT-PCR approaches. Recently, Markou *et al.* described a new liquid-bead-array hybridization assay (11).

In the CTC field, a very important biological question remains to be elucidated: the epithelial-to-mesenchymal transition (EMT).

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EMT is a complex process leading to cell dedifferentiation and increased motility via rearrangements of cellular contact junctions and eventually loss of cell adhesion. During this transition cells switch partially or fully their epithelial phenotype (i.e., expression of EpCAM, E-Cadherin, and CK) into a mesenchymal one (i.e., expression of N-Cadherin and Vimentin) and this switch has still an unknown impact on technologies identifying CTCs. However, as EMT might particularly affect tumor cells with stem cell-like properties (12), current assays based on epithelial antigens may miss the most aggressive CTC subpopulation (13). Thus, there is an urgent need for optimizing CTC detection methods through the inclusion of markers that are not repressed during EMT but still allow the analysis to distinguish CTCs from the surrounding blood cells.

Besides the choice of the appropriate CTC marker, the limited blood sample volumes available from cancer patients may impose a serious limitation on the detection of rare events such as CTCs, mostly in localized cancer with no overt distant metastases where the CTC counts are very low. Thus, technologies allowing the analysis of high blood volumes are of real interest and still deserve great attention. An elegant way to overpass this limitation is to target the CTCs directly *in vivo* via the Gilupi nanodetector®. During a 30 min application of this device in the peripheral arm vein, up to 1.5 L of blood, including the CTCs, pass the 2 cm functionalized area of the nanodetector and enable a high number of CTCs to be bound by the anti-EpCAM antibodies (14). An alternative approach is the development of leukapheresis, elutriation for subsequent *ex vivo* CTC analyses using flow cytometry and real-time PCR for molecular characterization (15).

Detection and molecular characterization of CTCs is one of the most active areas of translational cancer research with 427 clinical studies including CTCs as a potential new independent biomarker: CTCs reflect cancer progression in real-time and this information may be in particular helpful in the context of systemic therapies. The specificity and clinical utility of these CTC detection methods have to be demonstrated in large prospective multicenter studies to reach the high level of evidence required for introduction into clinical practice. In the future, CTC characterization will contribute to guide specific targeted therapies applied to a defined population of cancer patients at a certain therapeutic window, which is the hallmark of personalized medicine.

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