Liquid Biopsy and Colorectal Cancer

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Liquid Biopsies (LB) and their different methods have aroused great interest due to their potential possibility to be both sensible and specific markers for the diagnosis, prognosis and monitoring of cancers. Nowadays, only the CellSearch platform is a validated method for the enumeration of circulating tumor cells (CTCs) in metastatic breast, metastatic colon, and metastatic prostate cancers with the approval of the Food and Drug Administration (FDA) as a useful prognostic method. The clinical implementation of LB is not yet widespread.

Keywords: liquid biopsy ; personalized treatment ; cancers

1. Introduction

In the last decade, Liquid Biopsies (LB) and their different methods have aroused great interest due to their potential possibility to be both sensible and specific markers for the diagnosis, prognosis and monitoring of cancers. Nowadays, only the CellSearch platform is a validated method for the enumeration of circulating tumor cells (CTCs) in metastatic breast, metastatic colon, and metastatic prostate cancers with the approval of the Food and Drug Administration (FDA) as a useful prognostic method [1]. Despite current research to target the best methodology of LB for the purpose of diagnosis, prognosis or monitoring, several studies have focused on the supplemental aim of LB: adapting treatments as best as possible to provide a personalized choice that will help to avoid resistance to the treatments and therefore recurrences.

2. Liquid Biopsy (LB) and Targeted Approaches for Analysis

LB corresponds to the collection of liquids like blood, urine, cerebrospinal fluid, in which may be found tumor-derived material that is going to be analyzed [2]. In blood, we can detect different tumor products: tumor cells, nucleic acids, proteins, and exosomes. The two products that have aroused a lot of interest in the last 10 years are both circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) Circulating tumor RNAs, because of its fragility, remains the domain of very/high specialized centers.

All these products, coming from either the primary tumor, recurrences or metastases, are present within peripheral venous blood and can be studied even when there is no accessible tumor on which to conduct a biopsy. Contrary to tissue biopsies, without any danger for patients, blood tests can be performed at any moment and can be repeated at any time.

The first description of CTCs was introduced in 1869 by Ashworth T., an Australian pathologist who discovered them in the blood of a deceased patient and called this phenomenon carcinocythemia [3]. The origin of CTCs is linked to a phenomenon called Epithelial to Mesenchymal Transition (EMT).

To form distant metastases, cells must be able to detach from the tumor and enter the systemic circulation (intravasation) and then spread and form secondary tumors (extravasation) [4]. The cells therefore acquire the “stem cell” phenotype (and are cancer initiating cells, “CICs”) allowing them mobility, the basis of the metastatic process [5]. SNAIL, for example, is a zinc finger transcription factor and causes suppression of the expression of E-cadherin in different types of cancers such as breast, bladder, stomach and colorectal cancers. EMT thus allows fixed and polarized epithelial cells, which are linked laterally through several types of junctions and normally interact with the basement membrane, to undergo multiple biochemical changes: loss of cell adhesion, loss of “apex-base polarity”, cytoskeletal remodeling, acquisition of mesenchymal characteristics such as enhancement of migratory capacity, invasiveness, high resistance to apoptosis and increased production of extracellular matrix components.

This EMT process, which consists of the transformation of epithelial cells into mesenchymal cells with increased migration and invasion properties, appears to be regulated by “stem cells” signaling pathways [6]. Major signaling pathways including TGFβ, Wnt are thus involved in EMT and play a key role in tumor progression [7]. Various studies have also been able to demonstrate the role of the RAS–ERK 1-ERK 2 signaling pathway in the EMT of CRCs. Activation of the PI3K-AKT pathway throughPI3CAmutations or loss ofPTENis associated with colorectal tumor progression [8].

This phenomenon is so important that pharmaceutical companies are taking a very close keen interest in molecules that prevent this mesenchymal transformation into the epithelial phenotype. In addition, if there is no metastasis, there are at least circulating tumor cells. As long as these cells do not have to undergo the process of mesenchymal transformation to the epithelial phenotype, the formation of metastases can be avoided. For this reason, many drugs currently being tested to stop this transformation are being studied, suggesting the “reversibility of the EMT system”: The overall EMT process thus allows CICs to produce more differentiated progenitors but also to convert non-cancer initiating cells to CICs.

CTCs are present in very small quantities in the blood: 1 CTC for 107leukocytes/mL of blood. They are very rare, “embedded” in other blood nucleated cellular elements, white blood cells [9]. Therefore, due to this rareness, their detection always goes through an enrichment and a selection phase.

The methods used to lead to enrichment and detection are based on the immunological properties or biophysical properties of these cells. Each method has its own advantages and drawbacks.

Based on their physical properties, we can use:Concentration gradient, Oncoquick®,because CTCs have a higher density than other cellular types [10]. The main advantage of this technique relies on its independence from the presence of specific tumor cell markers. However, a drawback exists: some tumor cells are small and pass through filters [11].Electrical features specific to CTCs lead to their discrimination from other cells using dielectrophoresis [12]. As it is a label-free method, it sorts cells independently from cell membrane markers such as EpCAM and is very specific [13].

On the one hand, positive selection enrichment methods (CellSearch®[14], CellCollectorTM, Ephesia) are based on immunoseparation using magnetic beads conjugated to an antibody directed against specific antigens of tumor cells, called “tumor-associated cell surface antigens”, generally EpCAM and cytokeratins (CK8, CK18, and CK19). All the techniques mentioned above target the specific antigen of EpCAM (epithelial cell adhesion molecule), a specific marker of cancer cells. On the other hand, negative immunoselection methods (RosetteSepTM) [15] are mostly based on a system of depletion of non-tumor cells, which carry specific antigens (such as specific markers for leukocytes).

As CTCs present in the bloodstream encompass the heterogeneity of the tumor, in the function of the technology used to detect them, different phenotypes of CTCs can be sorted.

The prevailing advantage of CTC detection is to provide cells with their integrity, to obtain nucleic acids (DNA and RNA) of good quality and a material analyzable protein. The collection of CTCs allows all techniques adapted to cytology: immunocytology, fluorescence in situ hybridization studies (FISH), molecular biology techniques (DNA sequencing, PCR, RT-PCR, multiplex RNA) and live CTC cultures for pharmacodynamic testing. Thereby, by preserving the molecular identity of the main tumor, they allow a range of analyses including DNA, RNA, and protein levels, as well as functional ones. Moreover, they can surrogate the current methods of follow-up of CRC (with images) leading to earlier diagnosis of recurrences and reduce costs [16].

The main disadvantages linked to the detection of CTCs are the risk of false-negative and false-positive results. Indeed, due to heterogeneity, it is possible that sub-populations cannot be screened or are incorrectly screened. Moreover, CTCs are in low abundance and frail which does not help with their detection.

The story of circulating tumor DNA (ctDNA) is also an old one. Circulating free DNA (cfDNA) was first discovered by Mandel in 1948 in the blood of healthy patients [17]. It is a broader term that describes DNA that is freely circulating in the bloodstream, but is not necessarily of tumor origin, in contrast to ctDNA which is considered tumor-derived, fragmented DNA in the bloodstream without being associated with cells. Fragments of these nucleic acids can come from either primary tumor, metastases and/or recurrences present within the plasma and can be derived from different mechanisms:Either necrosis or apoptosis of tumor cells in plasma;Or excreted from tumor cells within a vesicle called an exosome;Or contained within tumor cells.

The detection of ctDNA leads to the highlighting of tumor-derived mutations [18], but can also reveal epigenic aberrations [19] such as structural variance, methylation, and DNA fragment lengths.

There are several important advantages to detecting ctDNA. It is more sensitive to detect disease burden. As with the CTCs, it allows the detection of minimal residual disease after curative treatment. Furthermore, it can predict acquired drug resistance and influence changes in treatment modalities.

Indeed, it can be contaminated by normal circulating DNA (false-positive) or not be enough sensitive enough to the type of cancer being detected due to specific mutations (false-negative). In addition, pre-analytical conditions are insufficiently standardized for correctly detecting ctDNA : anticoagulant use, duration, freezing and transport. Furthermore, it is worth noticing that functional assays cannot be performed.

Through these different methodological approaches to detect these two products, specific molecular information can be provided. Thus, molecular profiling of LB markers may participate in an adaptation of anticancer agents and biological therapies to tailor treatment as specifically as possible to avoid treatment resistance and cancer progression.

Beyond the interest of both the quantification of CTCs and ctDNA which have been correlated to the aggressivity or the recurrence of the disease thanks to several clinical studies [20], it seems of high importance to use these tools in a qualitative and targeted way. Indeed, a major interest is to determine genotypic and phenotypic heterogeneity of CTCs and ctDNA through molecular and cellular analysis to follow their dynamic changes during cancer management follow-up (with treatment or without) [21].

Different methods are able to increase CTC yield, thus facilitating in vitro drug screens on CTCs leading to treatment adaptation. Over the last decade, microfluidic systems such as Parsortix [22], CTC-iChip [23] and the Herringbone chip [24] have been a remarkable tool to enhance CTCs isolation yield from patients samples [25]. By this enrichment of viable CTCs, genomic analysis with drug testing can be performed effectively.

Recently, some teams have tested the possibility of enhancing CTCs yield by combining leukapheresis withLPCTC-iChi [26].

In a recent study, the authors conjugated TRAIL on the surface of nanoscale liposomes along with the adhesion receptor E-selectin (ES) which is able to recognize and bind to most of the leukocytes [27]. Then, selectins facilitate deep adhesion to selectin ligands on tumor cells and leukocytes in blood. The signal for cell apoptosis is consequently initiated. All in all, these nanocarriers liposomes, by targeting leukocytes, enable them to present TRAIL on their surface aiming at killing CTCs.

In a targeted approach, the sequencing of DNA allows us to outline different specific mutations. It can inform treatment in such situations where mutations are the potential target of different therapeutic agents, contrary to other techniques (NGS-based panels) where many candidates have been interrogated such as am-Seq (Tagged AMplicon deep sequencing), Safe-Seq (safe sequencing system) or CAPP-Seq (Cancer Personalized Profiling by deep sequencing) [28].

Different targeted methods are available. However, two new technologies have improved the sensitivity detection of ctDNA : Droplet Digital PCR (ddPCR) and Beads, Emulsification, Amplification, and Magnetics (BEAMing). These two methods are high sensitive, fast and relatively inexpensive [29].

Derived from the digital PCR, ddPCR utilizes a droplet generator to partition DNA into droplets using an oil/water emulsion. These droplets then have individual polymerase chain reactions. Its sensitivity can vary depending on the percentage of DNA analyzed, but it is around 1 in 10,000 [30].

Mutations in ctDNA are identified by using flow cytometry. Sensitivity varies between 1.6 in 10,000 and 4.3 in 100,000 [30].

3. Clinical Applications in Colorectal Cancer: Overview of the Current Literature

Colorectal cancer (CRC) is the third most common cancer worldwide. With an overall 5-year survival of 56%, it is the second leading cause of cancer death [31]. The specific 5-year survival varies according to the histological stage of the disease at the time of diagnosis, going from 90.8% for the local stages Tis or T1, to 69.5% when there is a loco-regional invasion (stages II or III), and 11.3% for metastatic stages or stage IV. Both sexes combined, it ranks second among cancer deaths, despite the notable progress made over recent decades, when the CRC death rate fell by 25% between the periods 1984–1988 and 2004–2008 [32].

Thus, the current burden of cancer treatment lies in the presence of minimal residual disease (MRD). Indeed, in the bloodstream, we find the accumulation of different phenotypic profiles: those of the primary tumor, and those of metastases, which are completely distinct from the initial specimen but also from themselves over time. For instance, in the study of Xu et al., which included 566 metastatic CRC patients, in 5% of cases,KRASin ctDNA from plasma was mutated whereas it was wild-type in the tissue [33].

Until now, both reducing the risk of recurrences and improving overall survival have been linked to the addition of chemotherapeutic agents aimed at the functions of pathological markers such as lymph node status and size of the tumor. thatKRAS NRASmutations are negative predictors of response to anti-EGFR treatment for metastatic CRC patients [34], as well as On the other hand, antibody anti-HER2 treatment represents a positive therapeutic option for patients who are HER2 positive [35]. mutation is another marker of predictive negative response to conventional chemotherapies [36].

Thus, it seems of high importance to follow and quantify over time the modifications of biomarkers in LB such as ctDNA and CTCs to detect resistance, monitor disease progression, and adapt treatments according to each specific tumor profile.

Herein, we review the recent studies that have been conducted over the last 5 years, focusing on the use of both CTCs and ctDNA for detecting resistance to treatments and/or disease progression and triggering personalization of treatments.

By using the terms “ctDNA” “CTCs” “resistance” “disease progression”, and “personalization of colorectal cancer treatment” we conducted a literature search within PUBMED EMBASE and ClinicalTrials.gov including all the articles such as randomized controlled trials and clinical trials in English, focusing on colorectal cancer and LB as potential tools to adapt treatments. They corresponded to four clinical trials: the Prospect phase-2 trial: NCT02994888 [37], a Phase 2 Single-Arm Clinical Trial: CRICKET: NCT02296203 [38], a Prospective Ancillary Study to the Unicancer Prodige-14 Trial: NCT01442935 [39] and a phase All these trials encompassed patients with metastatic colorectal cancers (mCRC) and the methodology only focused on ctDNA analysis except for the ancillary study where CTCs were also analyzed.

Consequently, liquid biopsy demonstrates anticipation of tumor progression, informing physicians about the timing to modify clinical treatments and change treatment strategies.

The Danish clinical trial of Spindler et al. [40], by assessing ctDNA, has contributed to outlining the importance of early prediction to treatment response by ctDNA contrary to information brought by tumor sample analysis. Similarly, the ancillary study of prodige 14 has demonstrated this anticipated response to treatment before potential surgery, that is to say with a curative intent and not only at a palliative stage such as the previous clinical trial.

They showed that patients with ctDNARASnon mutated before rechallenge had a partial response with longer progression-free survival contrary to those with ctDNARASmutated (median PFS of 4.0 vs. 1.9 months [p= 0.03]). Thus, screening of EGFR signaling pathway by LB may contribute to the best adaptation of selection of patients who are best able to benefit from a cetuximab rechallenge. To confirm all these results with robustness, a new trial is upcoming: the CHRONOS (Phase II Trial: Rechallenge with Panitumumab Driven byRASClonal-Mediated Dynamic of Resistance) study (NCT03227926), in which patients eligible for cetuximab rechallenge are eligible “only if a decrease of at least 50% in the fractional abundance ofRASmutations in ctDNA is evident at the time of rechallenge when compared with the time of progression to the first-line anti-EGFR–containing therapy”. according to time and space, leading to the prediction of the response to anti-EGFR treatment.

All in all, these findings suggest the idea that ctDNA analysis is of great interest for monitoring clonal evolution and guiding therapeutic decisions.

While different retrospective studies have demonstrated that the number of CTCs are linked to the prognosis of any kind of cancer (colon [41], lung [42], breast [43], gastric [44], or urothelial [45] cancers), herein we have emphasized the interest of clinical research studies with well-conducted methodologies (such as CT or RCT). Indeed, these studies have highlighted the interest of looking for targeted expression genes in CTCs. in 2018 [46], by measuring the mRNA expression of stem cell (ALDH1) and EMT (PI3Ka, Akt-2, Twist1) markers in CTCs, they showed that Akt-2 expression may predict PFS in mCRC patients receiving different standard and/or experimental treatments. These results have demonstrated that CTCs can be a tool of applied value for prognostic evaluation, but also for effective individual targeted treatments.

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