

Circulating Tumor Cells (CTCs): From Detection to Dissection

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Summary

The breast cancer represents one of the most prevalent cancers in the world. The outlook for patients diagnosed with breast cancer has been changing for the better over time, with several significant advances coming through in the past 10 years; among them, and after highlighting the importance of prevention and early diagnosis, relevant advances as a better understanding of the process through gene expression analysis, at the ability to use specific therapeutic targets, and a more advanced hormonal therapy played a more than significant positive role. Nevertheless, and despite the above mentioned improvements in treatment and prevention, breast cancer remains one of the leading causes of cancer mortality in women.

Approximately 90% of cancer mortality is due to the development of metastatic disease. The metastatic process involves the discharge of tumor cells from the primary tumor into peripheral blood (circulating tumor cells, CTCs) to a target organ [1]. Recently, the clinical implication of detection of CTCs in breast cancer has been recognized and accepted [2]. In fact, the presence of CTCs has been correlated with a worse prognosis in early and metastatic breast cancer. As a result of these investigations, it has been reported that the amount of CTCs in the blood of these patients is an independent prognostic marker for overall survival and progression-free survival (OS and PFS) [3].

However, despite the accepted role of CTCs in the prognosis of breast cancer, the detection of CTCs alone does not provide enough information. Although the changes in CTC's amount per blood unit might indicate sensitivity or resistance to an anticancer therapy and provides information about the prognosis of the disease, this information is in many cases not enough [4]. The question we need to ask is, "how can we eliminate these CTCs in order to combat the disease"

The "annihilation of CTCs", involves the knowledge of their biology. The molecular and phenotypic characterization of these CTCs could be the key to understanding the mechanism of resistance to administered therapies and the door to the development of novel cancer treatments that specifically target circulating tumor cells.

In this short communication, we would like to briefly discuss the relevance of the molecular characterization of CTCs. Firstly, as new tools to potential clinical use in the monitoring of breast cancer, and secondly as potential biomarkers to development of new therapeutic targets.

Similar but not identical

The existence of different subpopulations of CTCs has recently been accepted. These subpopulations have different abilities to survive and proliferate. According to previously reported data [4], only 0.01% of these CTCs may survive, having therefore the possibility to originate new metastasis. In addition, the existence of genetic differences between tumor masses (primary tumor and metastasis) and CTCs has been reported [5]. The presence of these genetic discrepancies has a direct impact on the effectiveness of treatments. In fact, the heterogeneity present between CTCs and the primary tumor could develop resistance to the principal therapeutic target, such as HER2, hormonal receptors EGFR. More importantly, this heterogeneity generates clones that are resistant to therapy and have a high metastatic potential.

One of the most important genetic changes detected in CTCs is related to the epithelial mesenchymal transition process or EMT [6]. The EMT process has been proposed as one of the mechanisms of CTCs survival. During the EMT process, adhesion molecule expression is altered and cells take on mesenchymal characteristics, becoming more elongated, plastic, mobile, and thereby potentially aggressive. The acquisition of these characteristics increases the resistance of CTCs to conventional anti-cancer therapies and therefore increases their ability to survive. Furthermore, the gaining of this mesenchymal or semimesenchymal phenotype involves the activation of important genetic pathways as well as several transcription factors, including the Snail/Slug family, Twist, δ EF1/ZEB1, SIP1/ZEB2, and E12/E47 [7]. Snail has also been linked to tumor grade, metastasis, recurrence, and poor prognosis. Snail family proteins also collaborate with other transcription factors to orchestrate concerted regulation of EMT. Recent research shows that expression of Slug and Twist is highly correlated to human breast tumor. Snail and Twist cooperate in inducing ZEB1 expression during EMT [7].

On the other hand, current findings show that microRNAs (miRNAs) are also master regulators of EMT. MicroRNAs are single-stranded, small, 20-22 nucleotide long, non-coding RNAs that modulate gene expression at the post-transcriptional level [8]. miRNAs have been implicated in regulating diverse cellular pathways and are commonly deregulated in human cancers. miRNAs play a key role in diverse biological processes, and they are responsible for guiding different cellular functions such as cellular proliferation and apoptosis; actually, a single miRNA could regulate the expression of multiple target genes [9]. More interestingly, the same miRNAs may work as oncogenes or tumor suppressor genes, depending on the context and the cell type. Therefore, miRNA could activate the "Master Regulators" of adaptive changes in CTCs [10]. These findings suggest that miRNA could modulate therapy because these molecules could affect a number

of relevant gene networks, leading to significant biological changes in phenotype and potentially a reduction in the emergence of therapy-resistant clones.

As a result of those advances in the knowledge of the biology of CTCs, we not only have been able to increase the understanding of the metastatic process, but it has also been accepted that the detection of these CTCs with different phenotypes from the primary tumor can be utilized as biomarkers for tumor prediction and early assessment of therapy response [11]. In fact, the CTCs can be used as liquid biopsy to confirm the persistence of the therapeutic target present in the primary tumor. Recently, several groups have demonstrated that some therapeutic targets, such as HER2, can be present in the primary tumor while the CTCs remain negative. The result could translate into a potential loss of effectiveness of bevacizumab [12]. Therefore, the establishment of genetic profiles of CTCs in response to current treatments could be used as complementary information to solid biopsy. For example, these additional analyses could explain why more than 45% of patients diagnosed with breast cancer will develop resistance to treatment. More importantly, the detection and simultaneous characterization of CTCs could provide us information about the evolution of the disease during follow-up, minimizing the risk of overtreatment. Also, the CTCs could be good indicators for the administration of correct treatment [13]. Hence, the utilization of both liquid and solid biopsies is essential steps for personalized medicine.

Heterogeneity is well established in breast cancer. In fact, Breast cancer has been classified into five categories, based in the molecular patterns: Luminal A and B, Human epithelial growth receptor type 2 (HER2), Basal like and claudin-low.

The heterogeneity is not only focused on the different subtypes of breast cancer (intra-heterogeneity), but different tumor cell subtypes can also be detected within the same patient (inter-heterogeneity). Inter-heterogeneity can be specially detected in CTCs, for example, the existence of a subpopulation of CTCs with stem cell-like characteristics such as very slow replication and resistance to standard cancer therapy [14]. It can also be detected in locally advanced breast cancer, for instance, the subpopulation of CSCs with a CD44 high/CD24 low cell surface marker profile was more resistant to cancer therapies (chemotherapy, hormone therapy, and radiotherapy) than the major population of more differentiated breast cancer cells. Therefore, in accordance with these antecedents, we could define three different types of heterogeneity: First, the inherent heterogeneity results in the growth of the tumor. Second, the heterogeneity caused by acquisition of genetic characteristics involves the dissemination process which activates cell survival pathways. The third type could be the heterogeneity present in clones in which drugs can put selective pressure on the tumor cells to adapt and change.

Therefore, it is the heterogeneity that is the principal obstacle to cure breast cancer. Consequently if we characterize the primary tumor but not the CTCs, the clinical utility of these biomarkers will be limited. In fact, several studies have demonstrated that the presence of CTCs or micrometastases do not imply a subsequent relapse [15]. Theoretically, one of the principal reasons to explain these discrepancies between the presence of CTCs and the clinical outcome, is the type of device used to isolate and detect these CTCs. Currently, most of the devices used to isolate CTCs are based on the presence of EpCam expression, such as in the case of Cell Search system

(Veridex, LLC, Raritan, NJ, USA). Several studies have demonstrated that the lack of correlation between CTCs and the

clinical outcome when using Cell Search system, can be due to the lack of sensitivity of this device to isolate different subpopulations of tumor cells because not all CTCs express EpCam. Consequently, these CTCs may not be captured [16]. This is important because, as mentioned previously, these markers change as the cancer progresses, so the captured cells obtained by using such devices may only represent a subset of cells shed by the tumor.

As a result of these findings, new devices have been recently developed with more sensitivity that can better analyze specific CTCs. This new generation of capture devices detects different subpopulations of CTCs, which indicate the heterogeneity present in the disease of the patient. One example of these new devices is CTC-iChip. This device does not use cancer cell markers on the surface of the CTCs to identify cells for capture. Instead, the iChip efficiently removes the normal blood cells, leaving behind viable CTCs.

On the other hand, of a particular relevance could be the molecular characterization of these CTCs in order to provide more accurate drug target treatments which require the development of more sophisticated devices. In addition, the genetic characterization of CTCs can provide insight into the future clinical behaviour of cancer, specifically in relation to target therapy.

In conclusion, we must establish genetic and phenotypic profiles of CTCs related to markers of aggressiveness and markers associated with current treatments to be used in the immediate clinical application of CTCs in breast cancer. This is because the liquid biopsy not only provides real-time information on the stage of tumor progression, but their phenotypic and genetic characterization is also a fundamental step in assessing the effectiveness of the treatment and cancer metastasis risk. For these reasons, advances in CTCs research must be linked to technological development.

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