EUR. J. ONCOL., vol. 19, Suppl n. 1, pp. 11-13, 2014

Circulating tumor cells: current challenges and future perspectives

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In last years, considerable effort has been focused on the identification of new reliable biomarkers, to improve stratification, monitor therapy response and identify potential new therapeutic targets for patients with GI tumors. More recently, CTCs are, potentially, the most easily observable event during malignancy, because of the non-invasiveness of venipuncture. The presence of CTCs correlates with an unfavourable clinical outcome in colorectal cancer and therefore enumeration of CTCs may be clinically useful as a prognostic factor (1).

Despite these great promises, the exact critical role of a specific number of CTCs in a fixed blood volume to define poor prognosis or good prognosis needs to be established. From a biological point of view it is logical to hypotize that the number of CTC present in the blood of a patient is greater the more aggressive the disease is, but this relationship has not still been definitively demonstrated. Hence, caution should be employed in clinical practice when using a threshold cut-off number to stratify patients into different prognosis subgroups. Also, several factors may influence the statistical accuracy of the different CTC assays, including efficiency with regard to the recovery/preservation of CTCs during sample preparation, quality of the starting sample, frequency of the events of interest, specificity of the chosen markers and intra-operator/inter-laboratory variability (2). However, given the variability associated with the so called "rare event" detection, additional research is needed to establish sensitivity thresholds and the methods employed need to be standardized in large, multi-institutional trials before such assays are widely applied. Devices for CTC analysis will allow a better definition for the biological role of CTCs during cancer progression however, isolating and molecularly categorizing CTCs is extremely challenging. Identification and separation of these cells (whose frequency is usually lower than 1-5% of total cells) can be achieved using specific panels of antibodies. However, recently it has been suggested that systems based on biological markers don't give a comprehensive phenotypic definition of CTCs, because classical epithelial and tumor markers are lost on some CTC populations during the epithelial-mesenchymal transition (EMT)-process (3).

The current development of devices for the analysis of CTCs is increasingly focusing on sensitivity, affordability, and the capability to manipulate tumor cells for the analysis of their genetic makeup, gene transcription, or biological behavior (4). In particular the molecular characterization of CTCs could give important contributes to our understanding of the metastasis phenomenon. The new emerging technological platforms that are particularly dedicated to this type of study still need significant optimization to enable a reliable isolation of viable CTCs before currently available molecular approaches may be largely applied. Label-free approaches to analyze CTCs have been recently invoked as a valid alternative to "marker-based" techniques. Several techniques for the label-free isolation of CTCs have been developed,

many of them rely on microfluidic approaches and can be divided into two major groups, based on their methods for detection and/or enrichment: a) differences between cellular biomechanical properties (e.g., cell size, density, deformability); b) differences in cellular electrical properties (5).

In this line of research and taking into account the difficulties of most technical approaches in the enumeration of "rare events" in large volumes of blood (as well as the limited capability to sort, under viable conditions, the cell subpopulation of clinical interest) we have developed an innovative and simple approach for the immunomagnetic enrichment of CTCs and the final capture of these cells in a central point of a glass slide suitable for conventional microscopic analysis (2). Our recently patented system (Micro-Count™) exploits the validated technology of magnetic bead labelling with the peculiar advantages of conventional microscopy in managing a sample composed by a very limited number of unlabelled cells. A few hundred cells on a glass slide can be: a) directly counted, in phase contrast for the first enumeration, b) rapidly tested by both bright field or fluorescence to check their viability, c) transferred to a vial for additional advanced molecular biology studies and, d) further morphologically investigated with the aid of higher magnification (100X) after labelling with a variety of immunofluorescent specific markers. The system is composed of a plate housing different types of highly efficient neodimio-magnets located in the bottom of a six well standard flask used for enrichment/separation. A square 20mm glass coverslip may be used to recover CTCs at the end of the procedure. This coverslip can be easily handled for further cytological characterization. Alternatively the separated cells can be directly recovered at the well bottom for successive molecular investigations. Peculiar advantages of the system are: a) there is no need to stress the sample inside the standard magnetic column where the "positive selected" cells must be released by mechanical shock, b) it is a no-wash / no-lyse procedure that guarantees reduced manipulation and minimal interference in cell viability, c) sample storage after counting for permanent case record in which further analyses is allowed.

The label-free approaches in the field of CTC-studies have been further explored when these devices are set-up in a controlled microspace where different physical and/or electro-physical processes are employed to specifically manipulate target cells under different conditions and as much as possible in a hands-off mode. Miniaturisation strategies together with nanotechnologies have been used to advance lab-on-a-chip for capturing, separating, enriching, and detecting different CTCs efficiently, while meeting the challenges of cell viability (6). Another hurdle is to achieve conditions able to minimize the interference of unwanted cells and concurrently to maximize the signal/s (either fluorescence and/or any other physical information). Several new microdevices have been specifically constructed to combine diverse established principles of analysis and detection in a "microspace", in order to guarantee an highly efficient approach to detect and differentiate the cell sub-population of interest.

In conclusion, although large-scale clinical data on how molecular characterization of CTCs may be used as a clinical decision making tool are still laking, this type of analysis holds important promise with respect to better biological understanding of the metastatic process, improved stratification of patients and, in the near future, establishing a genetic profile for each tumor (7,8). For these reasons, there is a great need for development of more sensitive technologies able to avoid stress on the cells during manipulation, in order to preserve cell viability and proliferation capability and to facilitate subsequent in vitro culture for further molecular analysis (9).

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