

After clouds sun again shines on circulating tumor cells research

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Abstract: In the Science issue of first February 2013 Yu M *et al.* characterized epithelial and mesenchymal circulating tumor cells (CTC) by RNA-in situ hybridization. In this editorial we comment their results and emphasize the different CTC subpopulations arising from epithelial mesenchymal transition (EMT).

Key Words: Circulating tumor cells (CTC); epithelial mesenchymal transition (EMT); Cancer stem like cells; mesenchymal epithelial transition (MET)



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Increasing evidence suggests that circulating tumor cells (CTC) are implicated in tumor metastasis. Although the risk of cancer death is linked to the disease stage, the most part of cancer deaths results from disseminated metastases (1,2). To detect early tumor cells dissemination numerous methods have been developed. Many research works are focused on CTC in the peripheral blood. The hematogenous transport phase of cancer cells is a particularly attractive opportunity for the follow-up of cancer. The blood stream represents the pathway of metastatic spread, even if cancer cells initially disseminate through lymphatic system which is connected to the general circulation. Therefore CTC provide a great potential for prognosis and monitoring of therapy response in epithelial cancers (3). Many approaches for enrichment, isolation and characterization of CTC have been published. Their multiplicity testifies of the weakness of their analytical qualities. Today none of the developed methods can be considered as the “Gold Standard”. This is partly due to the collectively used capture of cells by EpCAM antibody. This method cannot detect all cells which have heterogeneous characteristics. CTC are not a single population but a highly mixed one. They are constituted by subpopulations stemming from the epithelial mesenchymal transition (EMT). For the first time, we previously described, in a clinical trial analysis, these subset populations of CTC: epithelial, epithelial and mesenchymal, mesenchymal

endowed with stemness characteristics cells in the blood of patients at the early time of breast cancer diagnosis (4). EMT is a morphogenetic process, aberrantly activated in cancer, in which tumor epithelial cells lose apicobasal polarity, adhesion proteins, cobble-stoned histological aspect and epithelial characteristics including EpCAM expression.

In the February issue of Science, Min Yu *et al.* demonstrated that circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition (5). In an index patient, reversible shifts between these two cell populations accompanied each cycle of response to therapy and disease progression. To address technical challenges and reliance on epithelial markers they optimized microfluidic capture of CTC with epithelial and tumor specific antibodies. Thereafter they used this technology to analyze EMT in CTC from breast cancer patients. Authors provided evidence of EMT in human breast cancer specimens particularly in CTC. They found a striking association between expression of mesenchymal markers and clusters of CTC rather than single migratory cells. They underlined the role of platelets that release TGF β . This observation is supported by strong TGF signatures in mesenchymal CTC clusters surrounded by blood platelets (6). Despite the huge endeavor showed in this publication, analysis of CTC is still challenging. Effectively, it seems difficult to apply to common clinical

practice a methodology which is rather the privilege of research laboratories. Finally we would underline some points of this work. The main advantage of microfluidic devices is that they can handle sample volumes at least 10 times smaller than those used in other methods. Authors processed 3 mL of blood through their microfluidic device but they did not take into account that detection of CTC is always hampered by the problems of the Poisson statistics. The analysis of larger blood volume might be particularly preferable at the early time of diagnosis when a small burden of CTC is suspected. To overcome the limitations of small blood sample volumes of the *ex vivo* CTC isolation techniques, new approaches are needed to screen large blood volumes *in vivo*. Such a system able to isolate CTC across all tumor stages, including early stage cancer has been described by Saucedo-Zeni *et al.* In this method the volume of tested blood is estimated at 1.5 to 3 liters (7). The second point we want to delineate is the EMT process. EMT promotes local tumor invasion, intravasation and extravasation in the systemic circulation. But reversion of EMT, i.e. MET is essential in the establishment of macrometastases. Authors did not evoke the spatiotemporal regulation of EMT. In many types of cancers EMT is not activated in distant metastases. EMT model implies to take into account a level of cellular plasticity in the tumor cells. In their noteworthy paper authors did not write about the proliferative character of CTC as either single or clusters. Studies about Ki67 would be necessary. We supposed that single cells are more or less proliferative and that clusters cells are not. During tumor progression, we think that environmental cues in the primary tumor activate the EMT program. Along the migration to a distant site there is a progressive loss of EMT activating signals until the cells extravasate into the tissue parenchyma. During this evolution from “EMT on” to “EMT off”, cells are endowed with stemness characteristics and a new subset population has to be added to those described by Min Yu *et al.* This subpopulation is made of cancer stem like cells that can be characterized by markers like ALDH1, BMI1, CD44 or others. We showed in a clinical trial that these markers are increased in CTC and that they were associated to mesenchymal markers (8). So we cannot define pure CTC populations as the transient nature of EMT requires a fine balance between the maintenance and loss of epithelial characters leading to metastasis *in vivo*. A partial EMT would be sufficient to promote metastasis. Finally whatever

the status of CTC, is there a relationship between CTC abundance and the development of metastases? Therapeutic agents could inhibit EMT but due to the transient nature of EMT it could be better to block the MET thus avoiding transformation of mesenchymal cells into cancer stem like cells. Nonetheless the publication of Min Yu *et al.* is a major paper in oncology and open the way to new developments in CTC research, so far from just counting CTC in blood samples.

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