Circulating tumor cells (CTCs) were previously considered to be rare events difficult to identify. In clinical practice, CTC enumeration has now been recognized for its prognostic value. Furthermore, as CTC technology has rapidly improved, CTCs are now readily available for further investigation. The tumor cells isolated from CTC instruments may be analyzed for molecular profiles and mutation screening. Here we propose three new subsets of CTCs: elongated CTC (EL-CTC), miniature CTC (Mini-CTC) and cluster CTC (CL-CTC). Elongated CTC have an elongated shape and some of these cells have an enlarged tail composed of the nucleus. The enlarged tail is formed during intravasation because of the less flexible nuclear membrane and these cells represent highly motile tumor cells. The mini-CTCs are much smaller than regular tumor cells and may be dormant and gain the advantage of escaping immune surveillance. The CTC clusters are formed by a group of tumor cells and lymphocytes as well as macrophages. There is strong evidence that these clusters are highly malignant. These three subsets are recognized morphologically and their unique features may directly relate to their functionality and clinical significance. Additional investigation in the area of CTC research, specifically enhanced understanding of CTC subpopulations, may lead to novel therapeutic approaches in oncology.

Table 1. EMT review. Major progress in the study of CTCs in the past several years has focused on the role of EMT in tumor metastasis. Here is a summary of some features of this transition.

<table>
<thead>
<tr>
<th>Morphology</th>
<th>EMT-transformed Cells</th>
<th>Epithelial Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Elongated, spindle-like</td>
<td>Most are round shape</td>
</tr>
<tr>
<td>Molecular Profile</td>
<td>VIM, FN1, CDH2, SPARC, SERPIN1, ACTA2,......</td>
<td>CDH1, OCCLN, PKP2, GJB2, TJP3, KRT18,......</td>
</tr>
<tr>
<td>Motility</td>
<td>High</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Figure 1. This depiction shows a CTC cluster. Tumor cells are marked in green. The cluster may contain other cell types, such as lymphocytes (WBC, in blue), macrophages (in red), and dendritic cells (not shown). Some reports indicate that certain genes are up-regulated in clusters as compared to individual CTCs. In the CellSearch test every CTC cluster is enumerated as one CTC. CTC clusters may hold clinical significance related to augmented metastasis. Studies on the role of macrophages in metastasis indicate they may exert paracrine effects as metastasis facilitators. The exact role of lymphocytes is unclear. It may be interesting to assess PD1/PDL1 on those cells trapped within cluster.

Figure 2. Elongated CTCs have a distinct feature: tails composed by less flexible cellular nuclei (in purple, the CellSearch System from Janssen Diagnostics assigns this color to DAPI staining). These CTCs are highly motile, and may also represent a small population of epithelial-mesenchymal-transition (EMT)-transformed cancer cells.

Figure 3. CTCs present a diverse shape and size. Regular CTCs (left panel, A) are larger when compared to miniCTCs (right panel, B). MiniCTC can be up to ten-fold smaller than the size of regular CTCs. The CellSearch System provides a square box during the process of image review to define the minimum CTC size requirement (4μm). The smallest CTC shown here (first row of right panel) meets this requirement.

References


Contact: Dr. Chengsen Xue — chengsen.xue(at)iconplc.com and Thomas W. Mc Closkey, PhD — thomas.mccloskey(at)iconplc.com