



Commentary

Phenotypic heterogeneity in circulating tumor cells and its prognostic value in metastasis and overall survival



Kritika Saxena, Ayalur Raghu Subbalakshmi, Mohit Kumar Jolly*

Centre for BioSystems Science and Engineering, Indian Institute of Science, Bangalore, India

Liquid biopsy is fast emerging as a powerful tool to monitor and predict tumor recurrence and therapeutic response of patients [1]. Isolating and characterizing circulating tumor cells (CTCs) is a major effort in this direction, and CTCs have been shown to be good predictors of metastatic progression and therapeutic efficacy [1]. CTCs can be genetically [2] and phenotypically [3] quite heterogeneous; each subpopulation may contribute differently to metastasis, therapy resistance, and the consequent clinical course. However, the differential contribution of these subpopulations is not fully understood. Sun *et al.* investigate the phenotypic heterogeneity of CTCs in pancreatic ductal adenocarcinoma (PDAC) patients and demonstrate the ability of different CTC phenotypes in assessing tumor metastasis and overall survival (OS) of patients [4].

Isolating CTCs has been a challenging task, given their extreme rarity in circulation, compared to the number of blood cells [1]. Various methods based on physical properties or biological characteristics have been developed to isolate them and have identified heterogeneous subsets of CTCs [1]. But, the absence of any comprehensive analysis of CTC phenotypes from the viewpoint of various molecular mechanisms implicated in tumor aggressiveness leaves a gap in connecting the pre-clinical and clinical observations. Therefore, the functional role of varied CTC phenotypes in metastasis and drug resistance remains elusive. Sun *et al.* investigate CTC heterogeneity in terms of Epithelial Mesenchymal Transition (EMT) – a cell biological process implicated in metastasis, therapy resistance, and patient response, and quantifies the levels of epithelial (*E*-CTCs), mesenchymal (*M*-CTCs) and hybrid epithelial/mesenchymal (*H*-CTCs) in PDAC patients [4].

Recent *in silico*, *in vitro* and *in vivo* studies have identified the mechanisms maintaining cells in a hybrid epithelial/mesenchymal (*E/M*) state and showed that cells in this state can be much more tumor-initiating as compared to canonical epithelial and mesenchymal ones [5]. The prevalence of hybrid *E/M* CTCs in aggressive tumors has been reported previously too [6,7], but this study offers valuable insights by a quantitative comparison of the predictive prognostic power of *E*-CTCs, *M*-CTCs and *H*-CTCs in PDAC. The results reported here consolidate the emerging evidence from preclinical studies that hybrid *E/M* phenotype may be among the ‘fittest’ ones for metastatic progression [8].

The authors used extensive statistical modelling to analyse the ability of different combinations of CTC phenotypes in predicting tumor metastasis and OS, in the 46 pathologically confirmed PDAC patients whose venous samples were collected preoperatively. Blood samples from 45 non-PDAC healthy individuals were taken as controls. The authors found that the multivariable model comprising of disjunctively combined phenotypes: “*H*-CTC \geq 15.0 CTCs/2 ml OR *E*-CTC \geq 11.0 CTCs/2 ml” was an optimal predictor of metastasis. Moreover, the authors showed that OS for *E*-CTC < 11.0 CTCs/2 ml was 16.5 months, while that for *E*-CTC \geq 11.0 CTCs/2 ml was 5.5 months. In univariable analysis, the *H*-CTC was ranked the best when comparing the performance among *H*-CTCs, *E*-CTCs, *M*-CTCs and total CTCs (*E* + *H* + *M*) in terms of distinguishing between local and metastatic patients. Thus, the authors reported that the level of *H*-CTC was a better predictor of metastasis, while those of *E*-CTC was a significant independent predictor of OS. Put together, this analysis resonates with the observations made in PDAC mouse models that a complete EMT may be dispensable for metastasis [5].

The authors have designed a microfluidic device containing many triangular chips (TU-chip™) to capture CTCs of all three phenotypes using a size-based, label-free approach. Consequently, this chip can capture all CTCs independent of the levels of EpCAM (epithelial cell adhesion molecule). EpCAM is a common target marker used by CTC isolation methods, including CellSearch, the only FDA-approved method to isolate CTCs. Given that EpCAM is usually lost during EMT, many of *H*-CTCs and/or *M*-CTCs may be missed by EpCAM-based chips, possibly resulting into lower yield of CTCs [1]. Another advantage of using microfluidics-based approaches is that they provide an integrated system where isolation, immunofluorescence labelling and quantification of CTCs can be done simultaneously. Thus, microfluidic devices offer a valuable tool to dissect the phenotypic heterogeneity of CTCs and their association with metastatic burden and survival [9].

Microfluidic devices have also been valuable in isolating clusters of CTCs [10]; these clusters may bear a disproportionately higher metastatic potential as compared to individual CTCs [5]. However, the authors in this study did not find any clusters of CTCs, perhaps due to the chip dimensions and/or an even rare presence of CTC clusters in circulation relative to the number of individual CTCs. Future studies should focus on dissecting the heterogeneity in CTC clusters as well and designing CTC isolation methods that can quantify the phenotypic distribution of individual CTCs and CTC clusters together. These advancements may

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* Corresponding author.

 E-mail address: mkjolly@iisc.ac.in (M.K. Jolly).

facilitate the use of various liquid biopsy methods to mature into an accurate and reliable predictive tool that may be used preoperatively to complement traditional imaging techniques and to eventually factor in while designing therapeutic strategies in the clinic.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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