Single-cell profiling of circulating tumour cells: a great leap forward

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Much hope lies in the study of circulating tumour cells (CTCs) to provide further breakthroughs in cancer biology as well as better treatments for cancer patients. CTCs are rare, freely circulating epithelial cells originating from primary or secondary tumours. Only within the last 15 years has the technology been available to isolate them from whole blood in patients affected by cancer [7]. CTCs are thought to be an integral part of the metastatic process ultimately responsible for cancer death. Crucially, CTC enumeration is an independent adverse prognostic marker in several advanced cancers including breast [2], colon, lung and prostate [5]. However, it is not yet established which molecular signatures confer CTCs with an ability to form ‘successful’ metastases and which molecular signatures might predict clinical outcomes such as relapse/recurrence or response to certain systemic therapies. The low morbidity associated with collecting CTCs from patients makes them obvious research material, with the hope of developing predictive biomarkers. In parallel with CTC research, the recent demonstration of gene expression heterogeneity within solid tumours by deep exome sequencing [3] has questioned existing practices that rely on molecular phenotypes obtained from single biopsies as foundation for developing biomarkers. Scientists and clinicians working with CTCs are understandably interested in uncovering whether molecular heterogeneity might also be found in CTCs. The paper of Powell et al [6] entitled ‘Single cell profiling of circulating tumour cells: transcriptional heterogeneity and diversity from breast cancer cell lines’ published in *PLOS ONE* in May 2012 provides answers to this question as well as opening new directions in this research field.

Using their own custom-made immunomagnetic separation technology called the MagSweeper, these authors were able to successfully separate live CTCs with high purity from whole blood samples from 50 patients with breast cancer (20 with primary and 30 with metastatic disease). Within this patient cohort, 105 individual CTCs (up to five CTCs per patient) were subjected to an 87-gene expression profile assay, covering genes considered to play important roles in a variety of metabolic processes including metastasis, epithelial-to-mesenchymal (EMT) transition, cell proliferation and stem cell phenotype. In control experiments using single cells from seven breast cancer cell lines, the authors convincingly demonstrated that the MagSweeper did not affect gene expression itself nor cell viability. From these same cell lines, through hierarchical clustering, it was found that individual cells clustered by cell line designation, thus confirming reproducibility of their gene expression assay and the expected clonal phenotype associated with these cells.

The study’s key findings were that unsupervised hierarchical clustering based on a subset of 31 commonly expressed genes in CTCs subdivided these cells into two distinct clusters and that in one-fourth of the patients, their CTCs clustered into both groups. This provides good evidence of the existence of gene expression heterogeneity between CTCs from different patients and, in some cases, within the same patient. The former conclusion is unsurprising as one would not expect similar CTC gene expression profiles from different patients. The latter finding is however quite remarkable, and it would be very interesting to see what results the authors would have obtained if they had tested more than five CTCs per patient.

The decision to pursue single-cell (as opposed to pooled) CTC gene expression has important consequences. Owing to only picogram quantities of RNA, whole-genome microarray analysis is apparently not possible [6]. Indeed, even on the more manageable scale of an 87-gene array, expression

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data were only available for a subset of 31 genes (from the 87-gene array) across all CTCs, whereas individual cells from tumour cell lines faired much better, highlighting some striking but perhaps not unexpected differences in the levels of RNA transcripts between CTCs and cells growing in culture medium. A limiting factor in single CTC gene expression profiling might therefore be low signal-to-noise ratios encountered for expression of certain genes of interest. These same obstacles might also be met in gene mutational analyses or even protein biomarker studies performed on single CTCs. As seen from a different perspective, low detected gene expression may itself be highly informative, providing evidence that certain genes are in fact switched off during the metastatic process, particularly when in transit in the blood, which the authors allude to by noting the low incidence of ER/PR/EGFR/HER2 expression of CTCs compared with that obtained from matched patient tumour biopsies.

Notwithstanding the fact that CTC and primary tumour gene expressions are evidently not always identical, harnessing molecular profiling of single CTCs for the development of predictive biomarkers and companion diagnostics will be challenging. Insights into real-time relationships between gene expression profiles of individual CTCs and paired primary tumour, and the effects of drug therapy, are likely to be further explored in mouse xenograft studies [1]. However, in order to achieve necessary statistical power, translational studies investigating the predictive utility of single CTC profiling in humans will likely need to bolt on to large-phase three clinical trials, with data volume and cost implications.

Although the CTC detection approach adopted in this study exploited epithelial cell adhesion molecule (EpCAM) positivity and stipulated this as a necessary defining criterion of CTCs, there exists a body of literature summarised in a recent review [5] explaining that some CTC subtypes are EpCAM-negative, becoming so during EMT, which is a necessary step in the metastatic process. There is also evidence in cancer patients of circulating tumour micro-emboli (CTM), made in part of aggregates of circulating tumour cells [4], the significance of which is unknown. Further studies are warranted to clarify what cell surface markers confer the most biological and clinical relevance to CTCs and equally whether researchers should be extracting gene expression information from individual CTCs in conjunction with material derived from CTMs.

The above observations will not detract from the fact that the work of Powell et al is groundbreaking, both from

technological and conceptual points of view. In paving the way for gene expression analysis in CTCs at the *single-cell* level, they have broken with the common practice of pooling data from larger sets of cells [8], which carries the risk of averaging data where large differences might exist. A framework has been set up within which the heterogeneity of CTC gene expression, between and within patients, can be properly studied. Certain questions can now be addressed in greater depth, e.g. (1) within a pool of captured CTCs, which are those that have the make-up conferring an ability to form new metastases? How does this relate to the known molecular characteristics of the primary tumour? (2) In a given cancer patient, how do CTC gene expression profiles of large numbers of CTCs evolve over time and across treatments? (3) How might this inform the likely success or failure of a given treatment?

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